Macroalgae Biogas for the Isle of Man: the Effect of Co-digestion

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MACROALGAE BIOGAS FOR THE ISLE OF MAN

THE EFFECT OF CO-DIGESTION

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MATERIAL ABSTRACT

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Laboratory-scale trials on anaerobic digestion of *Laminaria digitata* were undertaken in batch mode. Sludge from a working digester was used as inoculum. Macroalgae was digested individually and co-digested with creamery waste or sewage sludge pellets. The co-digestates are wastes on the Isle of Man, which forms a case study in marine bioenergy. Macroalgae digested with inoculum produced 159.67 ± 6.69 ml biogas per g lyophilised mass added. Macroalgae significantly increased the total volume of gas, relative to controls. Relative to controls, co-digestion of macroalgae and sewage pellets (50/50, lyophilised mass basis) had no significant effect on total gas. However, this co-digestion significantly decreased total gas relative to macroalgae only, possibly due to a low C/N ratio. Co-digestion of macroalgae with a 50/50 mixture of creamery waste and inoculum produced significantly more than controls. The same co-digestion had a greater effect than digestion of macroalgae without creamery waste. A 50/50 mixture of creamery waste and inoculum produced significantly more gas in total than did a higher ratio of creamery waste to inoculum, when macroalgae was added. An increase from 1 g/L to 2 g/L lyophilised mass of macroalgae added to mixed creamery waste and inoculum had no significant effect on total gas. However, some results were affected by liquid backflow. The decline in gas yields between trials is thought to have been due to microbial changes in the inoculum. The biogas had low methane content and would require optimisation. Areas for further experimental work were identified. Additional considerations related to the potential for, and impact of, macroalgae bioenergy on the Isle of Man.

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AD	Anaerobic digestion/anaerobic digester	17
ANOVA	Analysis of variance	63
atm	Atmosphere	44
BMP	Biochemical methane potential	32
Ca	Calcium	106
CHP	Combined heat and power	35
CH_4	Methane	21
C+LD	Digester with creamery waste and *Laminaria digitata* (trial 2)	53
C+LD.A	Digester with creamery waste and 1 g/L *Laminaria digitata* (trial 3)	53
C+LD.B	Digester with creamery waste and 2 g/L *Laminaria digitata* (trial 3)	53
C/N	Carbon/nitrogen	2
COD	Chemical oxygen demand	27
CSTR	Continuously stirred tank reactor	27
DI	Deionised	50
d.p.	Decimal places	42
EfW	Energy from waste	44
FAME	Fatty acid methyl esters	19
FID	Flame ionisation detector	62
GC	Gas chromatography/gas chromatograph	61
g	Gram	2
GHG	Greenhouse gas	15
H	Hydrogen	43
hPa	Hectopascal (100 Pa = 1 millibar)	72
HRT	Hydraulic retention time	27
H_2S	Hydrogen sulphide	28
ICP	Inductively coupled plasma	45
IoM	Isle of Man	17
IPCC	Intergovernmental Panel on Climate Change	15
JOIA	Japan Ocean Industries Association	36
k	Rate constant	115
kg	Kilogram	21
$KMnO_4$	Potassium permanganate	49
kWh	Kilowatt-hour	35
L	Litre	2

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1 INTRODUCTION

1.1 Marine bioenergy

World energy sources include coal, oil, natural gas, nuclear and renewables. There is a need to reduce the use of fossil fuels, which are finite, emit the greenhouse gas (GHG) carbon dioxide (CO_2) and can be associated with geopolitical problems. The energy supply sector is the largest contributor to GHG emissions globally (Bruckner *et al*., 2014), and future supply and demand of energy must be considered. Mitigation of climate change, and stabilisation of global temperature, require total emissions of CO_2 to be limited and eventually to approach zero (Allen *et al.,* 2009; Meinshausen *et al.,* 2009). The European Union is committed to reducing its collective GHG emissions to 20% below 1990 levels, by 2020. Under the Renewable Energy Directive (RED), 20% of energy consumption generated, and 10% of transport fuels, should be from renewable sources by 2020 (European Commission, 2009). Renewable sources include biofuels, solar, geothermal, hydro, tidal, wind and others.

It is expected that generation and capacity of bioenergy will scale up significantly in the medium term (e.g. IEA, 2013). Biofuels are suggested likely to make important contributions to meeting heat and electricity demand in the longer term, and by 2050 could provide up to 27% of world transportation fuel (IEA, 2011). A biofuel is any hydrocarbon fuel that is produced by, or from, biomass (organic matter) in a short period of time (days, weeks or months). Fossil fuels, in contrast, form over millions over years. A wide range of bioenergy technologies are available, with various levels of maturity. The estimated shares in global final energy consumption in 2012 were 78.4% for fossil fuels, 2.6% for nuclear power and 19% for all renewables (0.8% biofuels) (REN, 2014). The end-2013 shares in global electricity production were 77.9% for fossil fuels and nuclear, and 22.1% for renewables (1.8% biofuels) (REN21, 2014). At the time of the Intergovernmental Panel on Climate Change (IPCC) Fifth Assessment Report, the global fuel balance for generation of heat was 46% from natural gas, 40% from coal, 5% from oil, 5% from biofuels and waste, and 2.4% from geothermal and other renewables, with nuclear contributing a small amount (Bruckner *et al.*, 2014). 3.4% of global demand for road transport fuel in 2012 was provided by biofuels (REN21, 2013).

Biomass fixes CO_2 during its growth, and CO_2 is released when the biofuels are used. However, there is a need to consider full energy supply chains on a lifecycle basis (e.g. Bruckner *et al.*, 2014). Environmental concerns related to bioenergy focus on land-use (direct and indirect) and

associated emissions of GHGs, deforestation, and potential competition with food supplies (e.g. Chum *et al.*, 2011; Smith *et al.,* 2014).

Marine biofuels are not usually based on existing arable crops (within Europe and the USA). Bioenergy production from land crops is expected to affect food prices, but it is thought that marine biofuels will not have the same impacts, due to there being less competition for areas of coastal biomass growth than for arable land (e.g. Bruton *et al.*, 2009). First-generation feedstocks are based on agricultural crops. Non-food sources are termed second- or third-generation feedstocks. Second-generation biofuels (the most developed being cellulosic ethanol) are produced using advanced technical processes. Third-generation biofuels (advanced biofuels or green hydrocarbons) cannot be distinguished from their petroleum counterparts. A likely feedstock for third-generation biofuels in future is algae.

Both micro- and macroalgae (section 1.2) are of interest as sources of liquid- and gaseous fuels. The conversion involved may use micro-organisms. Through their ability to capture CO_2, algae could potentially perform better in terms of net GHG emissions than other sources (e.g. Bruton *et al.,* 2009; FAO, 2010). Frequent examples show potential biomass yield per unit area to be higher for algae than for terrestrial plants (e.g. Tredeci, 2010; Walker, 2009; Williams and Laurens, 2010). Bruton *et al.* (2009) indicate the photo efficiencies (practical maximum) of microalgae and macroalgae both as 6%, compared with less than 1% for grass and for corn (Bruton *et al.*, 2009; Araya *et al.*, 2011). The energy potential of marine biomass has been estimated as over 100EJ per year, compared with 22EJ for terrestrial biomass (Chynoweth *et al.*, 2001). Productivities of microalgae and macroalgae are indicated as 20-75 (Bruton *et al.*, 2009) and 11-45 (Chynoweth, 2002) dry tonnes per hectare per year, respectively, compared with 12t/ha/y for grass (Murphy *et al.*, 2011) and 18t/ha/y for corn (Hirning *et al.*, 1987).

Microalgae are small and mostly single-celled. A key issue in studies on microalgae thus far is identification of species or types with optimal characteristics for fuel production, and much of the preliminary work concentrates on few species (FAO, 2010). *Macroalgae* are between a few millimetres and several tens of metres in size, and most of those used in energy production are tens of centimetres to several metres in size (Braune and Guiry, 2011). Only brown and green macroalgae, not red, have been used significantly as biofuels (Guiry and Guiry, 2010). Presently, it is thought that energy production from algae will need to be integrated with other high-value enterprises if the economic obstacles are to be overcome (e.g. Benzie and Hynes, 2013).

1.2 Algae

Algae are a group of photosynthetic eukaryotes. The two main types - microalgae (phytoplankton) and macroalgae (seaweed) - differ in their size and mode of life, and their properties have implications for their accessibility, culture and harvest (e.g. McHugh, 2003; Guiry and Guiry, 2010; Mata *et al.,* 2010, data as compiled by Benzie and Hynes, 2013). Today, microalgae are used in production of high-value food additives, materials for pharmaceuticals, biotechnology and cosmetics, and aquaculture feeds. Macroalgae also provide additives. Supplies and uses of macroalgae will be discussed further in section 1.3.3.

Biofuels from micro- or macroalgae are not currently being produced in economically viable quantities (Aresta *et al.*, 2005; Milledge and Heaven, 2014; Service, 2011). More research has been undertaken on the former, although it is noted that greater quantities of macroalgae (as wet tonnage) than microalgae are presently used in non-fuel applications (Lundquist *et al.*, 2010). For microalgae, research on energy production has concentrated on such fuels as bioethanol or biodiesel, with relatively few studies published on anaerobic digestion (AD) for biogas production (Sialve *et al.,* 2009). As lignin is not broken down in AD, its absence in algae is beneficial for this process. However, excess energy in microalgae is stored as lipids and oils instead of as sugars (Hu *et al.,* 2008). Whilst making microalgae suitable for oil and biodiesel production (Wijffels and Barbosa, 2010; Wijffels *et al.,* 2010), these high levels of lipids, together with the large amounts of protein also produced by some microalgae, can inhibit AD (Mata *et al.,* 2010; Sialve *et al.,* 2009). Macroalgae are better suited than are microalgae to AD and biogas production: they contain no lignin (e.g. Bruton *et al.,* 2009), their lipid levels are low (e.g. Bruton *et al.*, 2009) and their levels of fermentable carbohydrates are high (comparable to those in terrestrial sources) (e.g. Bruton *et al.*, 2009; Murphy *et al.*, 2011). Despite extremely high (logarithmic) growth rates coupled with a high lipid yield (Chisti, 2008), the main obstacle for the use of microalgae as biofuels is high cost of production (e.g. Benzie and Hynes, 2013; Carlson *et al.,* 2007). An additional factor is that in order to increase productivity closed photobioreactors are generally needed, particularly in more temperate latitudes. For cultivated marine macroalgae, production costs are much lower, but still much higher than those in agriculture and forestry (Carlson *et al.,* 2007).

The current project (outlined in section 1.5, section 1.6 and section 1.7) forms a case study of the Isle of Man (IoM) and will focus solely on marine macroalgae. The IoM has large amounts of beach-cast macroalgae, and harvesting costs would be covered if this was used in AD. More

information on macroalgae is given in section 1.3. The term 'algae', where used hereafter, will refer to macroalgae.

1.3 Macroalgae

1.3.1 General introduction to macroalgae

Macroalgae (seaweeds) are plant-like organisms. The majority are marine, but several species can grow in both marine and brackish environments (e.g. estuaries), or in landlocked freshwaters. Macroalgae contain several pigments involved in the capture of photosynthetically active radiation (PAR) and, based on this pigmentation, can be divided into three groups: red (Rhodophyta), green (Chlorophyta) and brown (Phaeophyta). Table 1.1 gives examples of macroalgae genera belonging to each of these groups.

Group	**Examples**
Rhodophyta	*Chondrus, Palmaria, Porphyra*
Chlorophyta	*Caulerpa, Cladophora, Ulva*
Phaeophyta	*Ascophyllum, Laminaria, Saccharina*

Table 1.1 Examples of genera representing each of the three groups of macroalgae

Unlike terrestrial plants, algae are non-flowering. Macroalgae lack the root systems present in terrestrial plants. The whole of a macroalgae structure is termed a thallus. A holdfast gives support. The leaf-like parts are called blades, and a collection of these is called a frond. A stem-like stipe may be present, and this transports nutrients to the holdfast. In order to attain maximum growth, macroalgae need to maximise the amount of light received by their chloroplasts. Macroalgae have chloroplasts in most surface tissues.

1.3.2 Chemical composition of macroalgae

The dry mass (dry weight or percentage dry weight) of macroalgae is related to products of photosynthesis. Given sufficient PAR, these products will accumulate in the cells. The main products that accumulate are complex polysaccharides, in addition to simpler polysaccharides, monosaccharides, proteins and, in lower amounts, lipids. Bruton *et al.* (2009) suggest dry mass in macroalgae to be 15%. The chemical composition of macroalgae varies due to several factors, including season (e.g. Redden, 2013). The main biochemical components of macroalgae are described next.

Lipids

Various methods of extraction can be used to determine the lipid content of macroalgae (e.g. Kumari *et al.*., 2011). As discussed by Redden (2013), the mass of lipids may be reported as free fatty acids, fatty acid methyl esters (FAME) or total lipids. Bruton *et al.* (2009) suggest lipids in macroalgae to be 0-2% of dry mass.

Protein

Various methods are used to extract and quantify protein in algae, and comparison of data can be problematic (e.g. Barbarino and Lourenço, 2005). However, protein in macroalgae has been suggested to be 12-19% of dry mass (Bruton *et al.*, 2009).

Saccharides

A wide range of structural and storage polysaccharides are found in macroalgae, and these are often particular to colour or species (Lobhan and Harrison, 1997). Bruton *et al.* (2009) suggest the total fermentable carbohydrates in macroalgae to be 20-60% of dry mass.

Structural polysaccharides in macroalgae are variable and complex (e.g. Percival and McDowell, 1967; Smith, 1991) and several have commercial value as phycocolloids, used as gelling and setting agents. The three main classes of gelling agents from macroalgae are agar (e.g. Zubia *et al.,* 2008), carrageenan (e.g. Dawes *et al.,* 1974) and alginate (e.g. Black, 1948abcd, 1950). Alginic acid, a polysaccharide composed of D-mannuronate (M) and L-guluronate (G) - two types of carbohydrates - can react with metal cations to form alginates (neutral salts) (e.g. Lewis *et al.*, 2011). Agar and carrageenan are obtained from different groups of red macroalgae, whereas alginates are contained in the cell walls of many of the larger brown macroalgae (kelps).

Each of the main macroalgae groups (red, green and brown) has characteristic storage saccharides. These include glucose, mannitol, starch, inulin and laminarin.

Ash

Ash is the non-degradable matter left after combustion of the biomass. Algae can contain 3.5-46% ash (dry mass basis) (e.g. Murphy *et al.*, 2013; Roesijadi *et al.*, 2010b; Ross *et al.*, 2008). It has been suggested that ash content is inversely related to carbohydrate content (e.g. Marinho-Soriano *et al.*, 2006).

1.3.3 Supplies and uses of macroalgae

For macroalgae, data on growth rates and productivity are scattered, and interpretation is complicated by differences between studies (Benzie and Hynes, 2013). However, a representative sample (Benzie and Hynes, 2013, from Bruton *et al.,* 2009; Habib *et al.,* 2008; Kelly and Dworjanyn, 2008) shows wide-ranging yields similar to, or greater than, those of terrestrial crops. Macroalgae can be harvested from the wild or grown commercially on a large scale (Werner *et al.,* 2004; Bird and Benson, 1987), or may be available as beach-cast. Dense populations of macroalgae have been successfully harvested (McHugh, 2003). In order for harvest to be economic, the quantity and growth of the algae must be sufficient. There may also be concerns related to sustainability of harvest. In addition to being more efficient to operate, and having lower costs and better mechanisation, aquaculture has the advantage (over wild harvest) of allowing culture of particular species and strains. Commercially, China is the main producer. McHugh (2003) summarises macroalgae culture.

A multimillion dollar industry is based on the growth and harvesting of macroalgae for various products (FAO, 2006). Much of the industry involves the extraction of alginates and gums, used as emulsifiers, thickeners and gelling agents. Other industry is based on nori (the genus *Porphyra,* used as food). Macroalgae have long been used as feed for domestic animals and as fertilisers, and provide additives used in the food industry and in nutrition. Some macroalgae are increasingly used in biosorption of substances including heavy metals (e.g. He and Chen, 2014) and nutrients (e.g. Reith *et al.,* 2004; Mulbry *et al.,* 2008). Conversion of macroalgae for use as a biofuel is considered in section 1.3.4.

1.3.4 Biochemical conversion of macroalgae

Composting

Composting (e.g. Mustin, 1987) involves fermentation of degradable substrates in aerobic conditions. This produces useable humic matter. The oxidation reactions are caused by living bacteria, fungi and animals. Given the proliferation of green macroalgae, which cannot be spread on soil raw, alternative methods of composting macroalgae developed (Brault *et al.,* 1985). Also driving this development was the composting of ligneous residues, as macroalgae could improve the composition of the substrate to be composted (Brault *et al.,* 1983; Potoky, 1983).

Fermentation to bioethanol

Fermentation involves conversion of sugars into ethanol and carbon dioxide, under anaerobic conditions. Macroalgae are better suited to production of bioethanol than of biodiesel, due to their relatively large amounts of fermentable sugars and relatively small amounts of lipids. Their relatively high water content (typically around 78-90%, e.g. Bruton *et al.,* 2009) can inhibit esterification of lipids, and dewatering adds to the cost of producing biodiesel (e.g. Benzie and Hynes, 2013). The yield of ethanol from fermentation varies but is usually 0.08-0.12 kg/kg dry macroalgae (Roesijadi *et al.*, 2010a). Horn (2000) demonstrated the viability of pilot-scale bioconversion to ethanol due to the large amounts of sugars (mannitol and laminarin) stored in macroalgae. It has been proposed that waste from the alginate extraction industry, which has these sugars as by-products, could be used in ethanol production (e.g. Benzie and Hynes, 2013).

Anaerobic digestion

Anaerobic digestion (AD) is the breakdown of organic matter (biomass) by micro-organisms, in the absence of oxygen. The process produces biogas and occurs in nature, but can also be used in an industrial system. The biogas is rich in methane (CH_4). AD of macroalgae is discussed further in section 1.4.

1.3.5 Thermal and thermochemical conversion of macroalgae

Depending on the process used, thermal or thermochemical biomass transformation can be used to generate heat, mechanical energy, or a product (liquid or solid) with a high energy content and in a suitable form for use as a combustible or as engine fuel. Four processes are outlined next, as applied to macroalgae.

Combustion

In combustion, the biomass is burned in the presence of oxygen, producing heat. Little research has been done on direct combustion of macroalgae (Wang *et al.*, 2013; Yu *et al.*, 2008). Although readily ignited, dry macroalgae have a low thermal value of 14-16 MJ/kg (Ross *et al.*, 2008).

Gasification

Gasification is an endothermic process in which organic matter undergoes partial oxidation at temperatures of 800-1000 °C and is converted mainly into syngas (Demirbas, 2001; McKendry,

2002a; McKendry, 2002b; Saidur *et al.*, 2011). The syngas is combustible, comprising 30-40% hydrogen, 20-30% carbon monoxide, 10-15% methane, 1% ethylene, and some nitrogen, carbon dioxide and water vapour (Demirbas, 2001; Saidur *et al.*, 2011), and has a calorific value of 4-6 MJ/m^3 (McKendry, 2002a). Achievement of gasification using wet macroalgae may be economically and energetically preferable to conventional gasification using dry biomass (e.g. Milledge *et al.*, 2014).

Pyrolysis

Broadly, pyrolysis is the heating of dry biomass in the absence of air, so that the organic matter undergoes thermal decomposition (e.g. Li *et al.* 2013; McKendry, 2002a; Saidur *et al.*, 2011). This results in chemically simpler products. Temperatures over 400°C can be applied to the biomass with the aim of producing charcoal, gas or pyroligneous liquor. An integrated plant designed and operated by Turrentine and Shoaff (1919), in the United States, was able to dry up to 100 tons of macroalgae per day and pyrolyse it at temperatures up to 980 °C. It is thought that the lipid content affects the energy balance of pyrolysis of microalgae, with higher lipid content meaning better energy content (Bhola *et al.*, 2011). Pyrolysis of macroalgae may therefore have a less favourable energy balance (e.g. Milledge *et al.*, 2014).

Hydroliquefaction

Liquefaction operates at low temperatures and high pressures, in the presence of a catalyst, and transforms biomass into a stable liquid fuel (Demirbas, 2001; McKendry, 2002). The process takes place in a reducing atmosphere created, for example, by synthetic gas (carbon monoxide and hydrogen). The temperatures used are around 300°C and the pressures are between 7 and 30 MPa (70 and 300 bar). Hydrothermal upgrading takes place in a wet environment, at high pressure and with a catalyst, and involves conversion of the biomass to partially oxygenated hydrocarbons (Demirbas, 2001; McKendry, 2002).

1.3.6 Evaluation of conversion methods

Composting is now well-developed, and the use of macroalgae can improve processing and the standard of the end-product. However, composting of macroalgae will probably remain local-scale for a long time (e.g. Morand *et al.*, 1991)

Direct combustion is suggested as feasible only if the biomass contains less than 50% moisture (McKendry, 2002a). However, direct combustion of dried *Ulva* has been suggested as a

relatively simple method, without the extra technological problems involved in liquid biofuel production (Milledge *et al.*, 2014; Yantovski, 2008). Given their low calorific value and relatively high moisture, ash and chlorine contents, macroalgae (if not pre-treated) seem unlikely to be suitable for combustion without system failure (Milledge *et al.*, 2014; Ross *et al.*, 2008).

Although the high content of metals, inorganic ions and moisture in macroalgae are disadvantages in combustion or pyrolysis, biofuel potential could be maximised by harvesting when metals concentrations reach their minimum and higher heating value reaches its maximum (e.g. Adams *et al.*, 2011a). However, storage is an issue. Combustion of macroalgae has supplied inorganic chemicals (e.g. iodine and potassium) for years. In contrast, pyrolysis is a promising method of making basic organic chemicals. However, although many useful products may be obtained, this requires the pyrolysis of very large quantities of macroalgae and the development of an efficient means of separating products. In general, this separation presents problems. While pyrolysis is technologically simpler than hydrothermal liquefaction (e.g. Schobert, 2013), the latter has the advantage of using wet macroalgae.

Gasification is much more rapid than AD, and a better energy balance may result if gasification could achieve a greater yield of combustible gases (Milledge *et al.*, 2014). It has been suggested that the technological challenges of treatment and refining to liquid fuels make thermochemical methods of processing macroalgae more applicable and versatile than AD and fermentation (Milledge *et al.*, 2014; Rowbotham *et al.*, 2013).

Fresh macroalgae has high water content (typically about 78-90%, Bruton *et al*., 2009) and drying forms the major cost in thermochemical conversion. The preference for wet algae (e.g. Horn, 2000; Murphy *et al.*, 2013) might suggest bioethanol production, hydroliquefaction or AD as more ideal conversion methods.

Lignin is problematic for many terrestrial biofuels, but as macroalgae usually have insignificant lignin content they have the potential for relatively easy conversion to bioethanol. Production of bioethanol is complicated by the fact that the biopolymers in macroalgae are not simple sugars (see section 1.3.2) and require specialised strains of fermenting bacteria. Red macroalgae, although less abundant worldwide than brown macroalgae, are relatively easily fermented, due to the lower alginate content of the former (Ha *et al.*, 2011). Using genes from the marine microbe *Vibrio splendidus*, Wargacki *et al.* (2012) engineered *Escherichia coli* so that

it could produce ethanol from brown macroalgae. In a project run by Bio Architecture Lab, a pilot-scale plant was built in Chile with the intention of demonstrating the feasibility of this ethanol-production process. However, it was decided that selling the raw material was better than processing it for fuel.

One benefit of hydrothermal upgrading is that it operates in a wet environment. However, it is thought that the energy balance is unfavourable given biomass with a moisture content of over 90% (e.g. Vardon *et al*., 2012, in relation to microalgae). The bio-oil (fuel) produced from hydroliquefaction is more stable than that from pyrolysis (Neveux *et al.*, 2014). However, it has been concluded that liquefaction involves more complex feed systems and larger costs than pyrolysis and gasification, resulting in low commercial interest (Demirbas, 2001; McKendry, 2002).

Bruton *et al.* (2009) suggest that although AD is the conversion method (for macroalgae) nearest to commercialisation, economic feasibility will require a reduction of at least 75% in the current cost of cultivated material. The cost of producing macroalgae biogas has been estimated as higher than the cost of natural gas (POST, 2011). More recent figures for cultivation are available. The idea of 'Ocean Food and Energy Farms' off the Californian coast was suggested in 1968 (Wilcox, 1982). This was tested in the US Marine Biomass Program during the 1970s and early 1980s (Chynoweth, 2002), but targets for bioenergy production (related to an oil crisis) were not reached and the program lapsed (given improved oil supply) in the 1980s. The only major-scale trial of bioenergy from macroalgae is by the Tokyo Gas Co. Ltd. (in Matsui *et al.*, 2006). The current thesis will focus on the use of AD for conversion of macroalgae to biogas, discussed further in section 1.4 and subsections.

1.4 Anaerobic digestion of macroalgae

1.4.1 Inoculum

An inoculum provides micro-organisms that are involved in AD. The sequence of AD is outlined in section 1.4.3. The inoculum in a digester is typically from municipal sewage sludge or animal manure slurry. However, in a study of AD of the macroalgae *Laminaria hyperborea*, Sutherland and Varela (2014) added bacteria from the rumen of sheep whose diet had consisted almost completely of macroalgae. Migliore *et al.* (2012) demonstrated the possibility of direct production of methane from *Gracilaria longissima* and *Chaetomorpha linum* using preserved spontaneous epiphytic micro-organisms to microbially start digestion, or using anoxic

sediments as a further inoculum. Miura *et al.* (2014) showed the feasibility of using marine sediments as microbial sources for methane production from *Saccharina japonica*. Schramm and Lehnberg (1984) noted that marine sediment gave a higher methane yield from green macroalgae than did non-marine inoculum. However, in a study using *Ulva* and *Gracilaria*, Costa *et al.* (2012) noted that anoxic marine sediment had no positive effect on methane production from batch assays. Although addition of marine bacteria can accelerate and increase biogas production, it has been noted that the final effect is no greater than that of co-digestion with slurries or traditional inoculum (Morand *et al.*, 1991).

1.4.2 Feedstock

Composition

Biodegradability is known to be related to composition (e.g. Bird *et al.*, 1990; Briand and Morand, 1997). The sequence of AD is outlined in section 1.4.3. The rate-limiting step in the process is thought to be the hydrolysis of polysaccharides, especially alginates (e.g. Sutherland and Varela, 2014). The main biochemical components of macroalgae are described in section 1.3.2. Differences in composition can have significant impacts on the performance and stability of digestion (e.g. Benzie and Hynes, 2013). A review by Forro (1987) showed that the main components of macroalgae tend to be readily degraded. Specific to macroalgae, Chynoweth (1980) noted greater biogas production from *Laminaria* when C/N ratios were low, whereas Habig *et al.* (1984), using *Ulva* and *Gracilaria*, saw an increase in biogas production with increased C/N ratio. Extraction of high-value additives (section 1.3.2) from macroalgae generates large amounts of residues that are rich in polysaccharides (e.g. Morand *et al.*, 1991). These wastes have also been trialled in digestion, as discussed in section 1.4.11 (e.g. Kerner *et al.*, 1991; Goes, 1988, cited in Morand *et al.*, 1991).

Pre-treatment

Macroalgal biomass can be dewatered (by mechanical methods) or dried to 20-30%, increasing 'shelf-life' and reducing transportation costs (Bruton *et al.*, 2009). It is thought that the rate-limiting step in AD is hydrolysis (e.g. Sutherland and Varela, 2014). Reducing the size of fronds before AD has been shown to significantly increase methane yields (e.g. Nikolaison *et al.,* 2012; Tedesco *et al.*, 2014). Some pre-treatments utilise the natural hydrolysis (percolation) of the algae (e.g. Brault and Briand, 1985a; Carpentier, 1986). Screening is required, although AD is very tolerant to foreign material. Sand and salt can be removed by washing, although results on the effect of salt on AD are mixed (section 1.4.9).

Co-digestion

Anaerobic co-digestion of two more types of organic substrate can have several benefits. Use of a co-digestate can increase biogas yields by creating positive synergetic effects in the digestion medium and supplying missing nutrients (e.g. Mata-Alvarez *et al.*, 2000). Besides carbon, nitrogen is the main nutrient needed for AD (e.g. Chynoweth *et al.,* 1987; Kelly and Dworjanyn, 2008). Optimum production of biogas from AD requires the maintenance of a balanced carbon/nitrogen (C/N) ratio (e.g. Deublein and Steinhauser, 2008). A C/N ratio of 20/1 of 30/1 is best (e.g. Kelly and Dworjanyn, 2008). The C/N ratio in AD is affected by several factors (e.g. Laura and Idnani, 1971; Schnurer and Jarvis, 2010), including substrate composition. Ghose and Das (1982, cited in Morand *et al.*, 1991) suggested that, in some cases, the biomethanation process could be improved by adjusting the C/N/P (carbon/nitrogen/phosphorous) ratios through the build-up of mixtures and co-digestion of substrates. N content of macroalgae has been linked to inhibition by ammonia (e.g. Costa *et al.* 2012, section 1.4.9). Marine biomass may be mixed with other feedstocks in order reduce the amounts of inhibitory compounds (e.g. Benzie and Hynes, 2013). Morand *et al.* (1991) noted that results from research on modification of C/N/P ratios involving macroalgae had proven negative. However, the limited amount of research on co-digestion of macroalgae has given mixed results (e.g. Kelly and Dworjanyn, 2008).

The mixing of wastes may also help to improve the N/P/K (nitrogen/phosphorous/potassium) ratio, thus increasing the value of the digestate as a fertiliser (e.g. Monnet, 2003). In some cases, co-digestion can help to establish the moisture content needed in the digester feed (e.g. Mata-Alvarez *et al*., 2000). In addition, co-digestion can simplify handling of mixed waste (e.g. Mata-Alvarez *et al*., 2000) and provide extra revenue from gate fees (e.g. Monnet, 2003). However, problems can arise from the costs of transporting slurry, and from differences between the policies of the waste generators (e.g. Mata-Alvarez *et al*., 2000). Given variations in the supply of macroalgae with season and time, and difficulties in large-scale storage of the biomass, co-digestion can serve to even out fluctuations in supply (e.g. Matsui and Koike, 2010).

Although co-digestion of macroalgae can have some benefits, results in the literature seem to be affected by the ratios of feedstocks in co-digestion and by the conditions of digester operation. Laboratory-scale co-digestion is discussed further in section 1.4.11, and large-scale co-digestion in section 1.4.12.

1.4.3 The sequence of anaerobic digestion

The process of AD involves a community of bacteria and can be considered in four main stages as follows:

A. *Hydrolysis.* The breakdown of large, complex polymers (such as carbohydrates, cellulose, proteins and fats) by hydrolytic enzymes produces simple sugars, amino acids and fatty acids.
B. *Acidogenesis.* Volatile fatty acids are produced from the breakdown of simple monomers.
C. *Acetogenesis.* Acetic acid is formed from the breakdown of the products of acidogenesis, releasing hydrogen and carbon dioxide.
D. *Methanogenesis.* Methanogens (a group of bacteria) form methane. This is done either by cleaving two molecules of acetic acid (producing carbon dioxide and methane), or by reducing carbon hydroxide with hydrogen.

1.4.4 Anaerobic digester types

An anaerobic digester can be batch or continuous. Batch digestion is simplest but can suffer from odour. Continuous reactors allow for more constant production of biogas and are more common. In continuously stirred tank reactors (CSTRs), the reactor contents are homogenised at all times. Digesters can also be classified according to temperature (section 1.4.7). In wet digesters, 5-15% of the matter is dry, whereas dry digesters contain more than 15% dry matter.

1.4.5 Solids retention time and hydraulic retention time

The period of time that the bacteria are in the digester is known as the solids retention time (SRT). The hydraulic retention (residence) time (HRT) is the length of time available for substrate digestion by the micro-organisms.

1.4.6 Organic loading rate

The organic loading rate (OLR) is the quantity of feedstock added per unit volume of the digester, per unit time. This is determined usually on the basis of total solids or volatile solids, but can be on the basis of chemical oxygen demand (COD). Improvement of AD, and prevention of failure due to overloading, requires information on process limits (e.g. Lindorfer *et al.*, 2008).

1.4.7 Temperature

There are two conventional operational temperature levels for AD: mesophilic (optimally around 37-41 °C or at ambient temperatures 20-45 °C with mesophile bacteria) and thermophilic (optimally around 50-52 °C or at elevated temperatures up to 70 °C with thermophile bacteria). A thermophilic process increases the rates of decomposition and biogas production, whereas a mesophilic process requires a larger reactor. However, due to their lower cost and greater stability, mesophilic digesters are still widely used.

1.4.8 pH

The sequence of AD is outlined in section 1.4.3. The pH influences the growth of anaerobes during all stages. The pH of the digester system is controlled by the concentration of volatile fatty acids (VFAs), the system alkalinity, and the fraction of CO_2 in the digester gas (McCarty, 1964). Although there are bacteria that are active within specific ranges, a digester will generally self-buffer (Gerardi, 2003, cited in Redden, 2013). If balanced, the system will tend towards pH 7 (Gerardi, 2003, cited in Redden, 2013). For the greatest biogas yield the optimal pH range in AD is 6.5-7.5, but the optimum value differs with substrate and method of digestion (e.g. Liu *et al.*, 2008).

1.4.9 Toxicity

Marine biomass may be mixed with other feedstocks in order reduce the amounts of inhibitory compounds (e.g. Benzie and Hynes, 2013). Co-digestion is discussed in section 1.4.2. An alternative is to use marine bacteria that may be more tolerant (e.g. Morand *et al.*, 1991). Inoculum is discussed in section 1.4.1. The 'acclimation factor' is important: the gradual introduction of a toxic substance into a digester creates significantly less perturbation than does sudden addition of that substance.

Sulphur

Macroalgae have high sulphur (S) content, usually 0.5-1% of dry weight (Show, 1985), and this level can be even higher in some macroalgae (Brault and Briand, 1985a; Show, 1985). Although S is needed for methanisation, it can also inhibit the process, specifically in the case of *Ulva* (Briand and Morand, 1997). Chen *et al.* (2008) review inhibitory concentrations noted in the literature as 100-800 mg/L for dissolved sulphide and around 50-400 mg/L for undissociated hydrogen sulphide (H_2S).

Metals

Heavy metals may cause inhibition where there are insufficient soluble sulphides to precipitate them (Morand et al., 1991). This problem does not occur given macroalgae with high sulphur content (e.g. Cecchi *et al.*, 1996) because sulphide precipitation happens naturally.

Salt

Salt might inhibit methanisation of macroalgae, depending on its sudden or gradual introduction to a reactor (De Baere *et al.,* 1984). Whereas low salinity can stimulate microbial growth, high salinity (≥ 10 g/L) is known to cause inhibition of anaerobic systems (e.g. Hierholtzer and Akunna, 2012; Kugelman and McCarty, 1965). 230mg/L has been suggested as an optimal concentration of sodium for mesophilic methanogens in waste treatment (Chen *et al.*, 2003). Acclimation of digesters to greater salinity is possible if salinity is raised gradually rather than there being a 'salt shock' (e.g. Lefebvre and Moletta, 2006; Lema *et al.*, 1988). Additionally, Hierholtzer and Akunna (2012) note that tolerance to salts can be greater when levels of ammonia are low. In some studies, salt has been shown to reduce mesophilic methanogenic activity (e.g. Chen *et al.*, 2003; Ramakrishnan *et al.*, 1998). However, removal of salt from macroalgae has been shown elsewhere to reduce methane production (Schramm and Lehnberg, 1984). Methane production has additionally been seen as lower in freshwater than in seawater (Schramm and Lehnberg, 1984). Other trials achieved stable digestion using a seawater system (Redden, 2013). Migliore *et al.* (2012) note that in salty environments various reactions and mechanisms may mitigate the impact of heavy metals. However, the methane yield from *Ulva lactuca*, for example, has been seen to be unaffected by washing of the algae (Nikolaison *et al.,* 2012).

Phenols

High concentrations of phenols can inhibit AD of macroalgae (e.g. Kelly and Dworjanyn, 2008). Due to its lack of lignin, macroalgae are lower in phenolic material than is terrestrial biomass (e.g. Ross *et al.*, 2008). However, macroalgae can adsorb phenols (e.g. Navarro *et al.*, 2008). This issue will be circumvented if phenols are pre-extracted due to their value as antioxidants (e.g. Matanjun *et al.*, 2008).

Volatile fatty acids

Volatile fatty acids are produced in acidogenesis (section 1.4.3) and their concentration is related to the stability of AD (e.g. Al Seadi *et al.*, 2008). VFAs can affect pH (e.g. McCarty, 1964)

and high concentrations can inhibit AD of macroalgae (e.g. Kelly and Dworjanyn, 2008). 4000 mg/L tVFA has been suggested as an upper limit for safe digester operation (Drosg, 2013).

Ammonia

The main forms of ammonia nitrogen in aqueous solution are ammonium (NH_4^+) and free ammonia (NH_3). Ammonia concentrations from 1.7 to 14 g/L have been reported as inhibitory to methanogenesis (Chen *et al.*, 2008). Given high levels of nitrogen in macroalgae, ammonia can accumulate and cause inhibition (e.g. Costa *et al.* 2012).

1.4.10 Expected outputs from anaerobic digestion: general considerations

AD produces biogas, a solid digestate and liquid supernatant. Typically, 50-80% of biogas is comprised of methane (CH_4) and around 20-50% of carbon dioxide (CO_2), with traces of other gases (e.g. hydrogen, carbon monoxide, nitrogen, oxygen, hydrogen sulphide). The digestate (material remaining after digestion) can be rich in nutrients. Depending on the substrate, this has potential for use as a fertiliser and for addition of revenue to the AD process (e.g. Roesijadi *et al.*, 2010). The use of macroalgae biogas has the potential for lower GHG emissions than those from natural gas use (e.g. Florentinus *et al.,* 2008).

As reviewed by Benzie and Hynes (2013), trials on AD of macroalgae have mostly been experimental-scale, using tank volumes ranging from less than 1 litre to several tens of cubic metres, and most pilot studies have been only several weeks or months long. Most experimental work has concentrated on the use of CSTRs with overall retention times between 1 and 60 days (mainly 20-60 days), and it has been noted that the state-of-the-art has advanced little since a review by Chynoweth in 2002 (Benzie and Hynes, 2013).

The volume and composition of biogas generated from AD will be affected by factors including the origin of the material, the environmental conditions, and the nature of the inoculum and the fermentation conditions (e.g. Briand and Morand, 1997). Table 1.2 shows selected values for potential biogas yields from common feedstocks in AD. It has been suggested that the C/N/P ratio should not be relied upon for estimation of the theoretical biogas yield from macroalgae, but that C/P and C/N ratios can be used in comparison of samples of the same species (e.g. Morand *et al.*, 1991). The theoretical biogas yield can be determined using the Buswell equation, given the composition of macroalgae in terms of C, H, O, N and S (Buswell *et al.*, 1952;

Symons and Buswell, 1933). However, while theoretical yields can be high, in practice the yields are much lower. Previous studies on AD of macroalgae are discussed next.

Table 1.2 Selected values showing potential biogas yields from common feedstocks in anaerobic digestion. Numbers (available via http://www.biogas-info.co.uk/biogas-yields.html) are taken from an AD calculator tool produced by the NNFCC and The Andersons Centre

Feedstock	**Dry matter %**	**Biogas yield m^3/tonne**
Cattle slurry	10	15-25
Pig slurry	8	15-25
Grass silage	28	160-200
Whole wheat crop	33	185
Maize silage	33	200-220
Maize grain	80	560
Crude glycerine	80	580-1000
Wheat grain	85	610
Rape meal	90	620
Fats	Up to 100	Up to 1200

1.4.11 Previous work: laboratory-scale anaerobic digestion of macroalgae

Laboratory-scale mono-digestion of macroalgae

Different studies have different ways of testing and reporting growth rates, productivity and biogas yields of macroalgae, and Benzie and Hynes (2013) note the difficulty of summarising available information. Together, trials carried out on macroalgae in the early 1980s demonstrate robustness over a range of species and conditions, with biogas comparable in yield and quality to that from terrestrial biomass (Benzie and Hynes, 2013). Results from later research are similar (e.g. Vergara-Fernández *et al.,* 2008).

Research on AD of macroalgae has concentrated on various species, to greater or lesser degrees. Example species trialled belong to the genera *Ulva* (e.g. Bruhn *et al.*, 2011; Nikolaison *et al.*, 2012), *Gracilaria* (e.g. Bird *et al.*, 1990; Migliore *et al.*, 2012), *Sargassum* (e.g. Bird *et al.*, 1990; Oliviera *et al.*, 2015), *Macrocystis* (e.g. Ghosh *et al.*, 1981; Vergara-Fernandez *et al.*,

2008), *Laminaria* (e.g. Adams *et al.,* 2011b; Hinks *et al.*, 2013), *Palmaria* (e.g. Jard *et al.*, 2012; Jard *et al.*, 2013), *Saccharina* (e.g. Jard *et al.*¸ 2012), *Fucus* (e.g. Barbot *et al.*, 2015) and *Ascophyllum* (e.g. Hanssen *et al.* 1987), among others. Morand *et al.* (1991) carried out tests on over fifteen species of red, green and brown macroalgae, with the data together showing rates of biogas production between 0.11 and 0.31 m^3 CH_4/kg VS (volatile solids). The biogas was 50-67% CH_4. Redden (2013) trialled AD of nine macroalgae species. Mean CH_4 produced ranged from 0.02 to 0.10 L/g lyophilised mass, and methane content ranged 24.9 to 40.8%. Roesijadi *et al*. (2010b) noted that methane yields of 0.14-0.40 m^3/kg of VS had been reported from AD of macroalgae. Allen *et al.* (2015) assessed the biochemical methane potential (BMP), ultimate analysis and theoretical yields of ten macroalgae species from around the Irish coastline. The highest BMP yield (ca. 342 L CH_4/kg VS) was from *Saccharina latissima.* In a recent review by Chen *et al.* (2015), methane yields from 0.12 to 0.48 m^3/kg VS were noted.

Kerner *et al.* (1991) carried out lab-scale AD of waste sludges produced in industrial alginate extraction. Sludges were from the species *Laminaria digitata* and *Ascophyllum nodosum.* CH_4 production from batch trials was 0.10 to 0.15 L/g VS added, whereas production from semi-continuous fermentation was 0.07 to 0.28 L/g VS added.

Laboratory-scale co-digestion of macroalgae

The main results of previous co-digestion studies are outlined below, but results seem to be affected by factors such as the ratios of co-digestates and the conditions of digester operation.

Due to differences between the rates at which different macroalgae species digest, co-digestion of mixed species can be problematic. As metabolites are released, methanisation of one species could inhibit that of another (e.g. Jacq, personal communications cited in Morand *et al*., 1991). However, the addition of a small amount of *Ulva* to *Sargassum tenerrimum* has been shown to accelerate and increase biogas production (cited in Morand *et al*., 1991).

Oliveira *et al.* (2014) undertook BMP tests on co-digestion of *Gracilaria vermiculophylla.* Co-digestion with glycerol increased the BMP by 18% (giving a yield of 599 ± 25 L CH_4/kg VS) and co-digestion with sewage sludge increased the BMP by 25% (giving a yield of 605 ± 4 L CH_4/kg VS) relative to mono-digestion of the macroalgae. Oliviera *et al.* (2015) investigated co-digestion of *Sargassum sp.* with glycerol and waste frying oil. The BMP of *Sargassum sp.* was 188 L CH_4/kg COD. Co-digestion with glycerol and waste frying oil increased this by 56% and

46% respectively. The co-digestates increased methane production rate by 38% and 19% respectively. Costa *et al.* (2012) assessed BMP of co-digested *Ulva sp.* and waste activated sludge (WAS) in batch assays. The methane production rate was 26% greater than that from mono-digestion of WAS, with no decrease in overall biodegradability (42-45% CH_4 yield).

Pake *et al.* (2015) co-digested macroalgae (*Chaetomorpha sp.* and *Ulva intestinalis*) with natural rubber latex serum waste. The biogas yield from mono-digestion of the serum was 398 ± 14 L/kg VS added (VSA). The highest biogas yields obtained from co-digestion of the macroalgae with the serum were 422-460 L/kg VSA. For the optimised system over 45 days, the methane yield was 197 ± 16 L/kg VSA. In batch tests, Vivekanand *et al.* (2012) co-digested *Saccharina latissima* with steam-exploded wheat straw. Untreated macroalgae had a methane yield of 223 ml/g VS and pre-treated macroalgae produced 260-268 ml CH_4/g VS. Wheat straw had a yield of 98 ml/L CH_4/g VS. The overall methane production from co-digestions was greater the sum of mono-digestions of the feedstocks. The main effect was increased degradability of the straw, although addition of the straw did not give significantly lower methane production than mono-digestion of the macroalgae. Ramanathan *et al.* (2013) co-digested four species of macroalgae with a mixture of slaughterhouse waste and cow dung (or with a mixture of slaughterhouse waste, cow dung and cyanobacteria).Tedesco *et al.* (2013) carried out batch co-digestion of five macroalgae species with digester sludge. Allen *et al.* (2013) trialled co-digestion (BMP assays in batch mode) of *Ulva sp.* with dairy slurry. The highest specific methane yields were from 75% fresh *Ulva* (220L CH_4/kg VS) and 75% dried *Ulva* (210 L CH_4/kg VS). The methane yield from co-digestion was 17% greater than the sum of mono-digestion of the two feedstocks. However, the greatest yield on the basis of m^3 CH_4/t was 203 m^3 CH_4/t (from 75% dried *Ulva*). Morand *et al.* (1991) report that Carpentier (1986), using alginate extraction residues mixed with manure, observed lower production of biomethane than was shown by the corresponding calculations.

Further to work by Allen *et al.* (2013), Allen *et al.* (2014) undertook continuous long-term co-digestion of *Ulva lactuca* with dairy slurry. With 75% *Ulva*, stable digestion was difficult to achieve, but under optimum conditions (with 25% fresh *U. lactuca*) a yield of 170 L CH_4/kg VS was obtained. All digesters operated at steady state and at optimal OLR produced biogas with a methane content of 49% ± 3% (Allen *et al.*, 2014). In a lab-scale study using continuous reactors, Sarker *et al.* (2012) co-digested cattle manure with each of *Laminaria digitata* and *Ulva lactuca.* Mesophilic co-digestion of *L. digitata* was fairly stable, giving an average methane yield of 138 L CH_4/kg VSA. Thermophilic co-digestion of each of the species showed variation in methane production with changing loading rate, with an average yield of 142 L CH_4/kg VS from

L. digitata and approximately 122 L CH_4/kg VS from *U. lactuca*. Sarker *et al.* (2014) investigated mesophilic and thermophilic co-digestion of *Laminaria digitata* with cattle manure in semi-continuous digesters. In a lab-scale CSTR, Nielsen and Heiske (2011) co-digested cattle manure with dried *Ulva lactuca*, showing an increase in weight-specific CH_4 yield but a decrease in specific CH_4 yield as compared with mono-digestion of manure. Digester performance was enhanced. Nkema and Murto (2013) efficiently co-digested wheat straw with macroalgae hydrolysate in upflow anaerobic sludge blanket (UASB) reactors. The yield remained almost constant at 22 m^3 CH_4/kg COD as OLR increased. The methane yield from the co-digestion was similar to that achieved in the methane potential batch test. A yield of 0.19 m^3 CH_4/kg VS was obtained from mono-digestion of macroalgae hydrolysate (Nkema and Murto, 2013). Briand Morand (1997) co-digested *Ulva sp.* with liquid bovine manure, in completely stirred digesters. Although addition of algae to manure increased the rate of methane production per unit volume, total production was less than the sum of individual contributions from the feedstocks. It was noted by Briand and Morand (1997), however, that Rao *et al.* (1980) achieved effective and stable co-digestion of *Ulva* with cow manure, and that Rye (1988) obtained a yield of 0.50 m^3 CH_4/kg VS (62% CH_4) from co-digestion of *Ulva* with waste water sludge. Peu *et al.* (2011) co-digested beach-cast *Ulva sp.* with pig slurry, in pilot-scale digesters in the laboratory. The methanogenic potential of the macroalgae was 148 N m^3 CH_4/t VS (19 N m^3 CH_4/t crude product). Although co-digestion with pig manure did not notably disrupt digestion, the biogas contained up to 3.5% H_2S.

1.4.12 Previous work: large-scale anaerobic digestion of macroalgae

For practical operations, the AD process would need to be robust to changes in production parameters and composition of the feedstock (e.g. Benzie and Hynes, 2013). The early CSTRs have been suggested as inappropriate for larger scale energy generation, because the necessary high loadings would reduce conversion of biomass and cause instability (Chynoweth *et al.,* 1987). In contrast, vertical flow reactors (VFRs), when used in trials, gave a CH_4 yield of over 75% (Chynoweth *et al.,* 1987). Newer commercial CSTRs address some of the issues of previous designs, and have yet to be used on macroalgae (Kelly and Dworjanyn, 2008).

Large-scale mono-digestion of macroalgae

As yet, there is no ongoing industry producing macroalgae biogas. However, the Tokyo Gas Company (in Matsui *et al.,* 2006) undertook pilot-scale digestion of *Laminaria* (with a maximum biogas yield of 22 m^3 CH_4/ton) and *Ulva sp.* (which produced 15-17 m^3 CH_4/ton). Residues from

agar production have been trialled as a feedstock for biogas production (Goes, 1988, cited in Morand *et al.,* 1991) and developed further by Sopex (Belgium). An 800 m^3 digester was completed and used to treat 12 t of this waste (from Morocco) per day. However, figures on the operational efficiencies of the plant are unavailable. Large upscaling of digestion of macroalgae is therefore feasible, but has yet to provide long-term operational data (e.g. Benzie and Hynes, 2013).

Large-scale co-digestion of macroalgae

In a pilot-scale trial, Cecchi *et al.* (1996) investigated co-digestion of sewage sludge with macroalgae (mainly *Ulva rigida* and *Gracilaria confervoides*) from the Venice lagoon. Although there were differences given different operational conditions, and a limit for safe operation was pointed to, stable digestion was achieved, with performance comparable to that for sludge only. However, with thermophilic conditions digester failure was caused by abnormal H_2S levels in the biogas. Matsui and Koike (2010) undertook pilot-scale co-digestion of *Laminaria* species and *Ulva sp.* with milk, achieving largely stable methane production (0.2-0.3 m^3 CH_4/kg COD). The authors undertook lab-scale trials for comparison, and these also showed stable production. Biogas yields from the pilot-scale plant nearly reached levels expected from results of the lab-scale trials.

1.4.13 Energy generation

The amount of energy produced from biogas varies depending on the feedstock (section 1.4.2) and digester type (section 1.4.4). Biogas can be combusted to produce heat, electricity or both. Combustion to produce heat alone can convert 1 m^3 of biogas containing 60% CH_4 to 6.7 kWh (kilowatt-hour) of thermal energy (NNFCC, 2015). Electricity can be the most profitable form of generation from biogas and is a relatively straightforward use. However, storage is not simple and connection to the electricity network is costly. In addition, the efficiency of the gas engines that are usually used for direct generation is poor (less than 40%). The AD process requires some heat and so is suited to generation of combined heat and power. Overall efficiencies of over 70% at the point of use can be reached by CHP plants, compared to efficiencies of about 34% and 55% for coal- and gas-fired power stations, respectively (NNFCC, 2015). Generally, CHP plants convert 30-35% to electricity and 40-45% to heat, although the heat/power ratio varies according to the scale and technology (NNFCC, 2015). The biogas produced from large-scale AD by Tokyo Gas Co. (in Matsui *et al.*, 2006) was mixed with city gas and used to provide CHP. Over the past few decades, biogas has become important as an alternative to

conventional energy sources worldwide, particularly in developing countries such as China and India (e.g. Khoiyangbam *et al.*, 2011). Biomethane (pure methane) can be produced by biogas 'upgrading' (removal of other gases) and injected into the gas grid or used as a road fuel. However, inefficiencies in internal combustion energy must be considered in the economics of producing transport fuel from biogas (e.g. Kelly and Dworjanyn, 2008).

1.4.14 The potential for anaerobic digestion of macroalgae in the United Kingdom

The UK water industry, which has used AD for many years, currently treats 66% of sewage sludge from the UK by this method. There is now rapid growth in AD beyond this industry, and the UK is producing bioenergy from around 100 other digesters (NNFCC, 2015).

In the UK, AD could potentially produce 10-20 TWh (terawatt-hours) of heat and power per year by 2020, making up 3.8-7.5% of the renewable energy estimated to be needed in the UK in 2020 (NNFCC, 2015). There is, however, some disparity in uptake of AD across European Union countries and regions.

Currently, most algal biomass used to generate biogas is macroalgae harvested from the wild, and from eutrophic areas of estuaries and coastal zones where it grows unwanted (Bruton *et al.,* 2009). AD of macroalgae undertaken in Morocco, France and Japan was reviewed by Kelly and Dworjanyn (2008), who concluded that biogas production is viable under practical conditions. The Japan Ocean Industries Association (JOIA), based on core trials from 1980 to 1983, progressed the idea of a practical operation, concluding that economic feasibility requires the high-value by-products to be made (JOIA, 1984). The only major-scale trial on AD of macroalgae is that by the Tokyo Gas Co. Ltd. (in Matsui *et al.,* 2006). Macroalgae are an abundant and readily biodegradable renewable resource that can be a good feedstock for methane production (e.g. Chynoweth, 2002). However, seasonality of growth and cultivation is an issue for commercial-scale biofuel production. There is a need to develop methods of storage or preservation in order to meet demand of a continuous process, but limited work has been done on this (e.g. Black, 1955; Wout *et al.*, 2013).

The main uses of macroalgae are outlined in section 1.3.3. The biorefinery concept involves co-production of a range of commodities from biomass (e.g. Taylor, 2008). Biorefineries could improve the economics of bioenergy production from macroalgae (e.g. Bruhn *et al.*, 2011; Jung *et al.*, 2013), but extraction of alginate, laminarin and fucoidan removes compounds that would

otherwise be fermented in producing energy from the biomass (e.g. Bruton *et al.*, 2009). Potential saturation of the world market for phycocolloids is another issue (e.g. Bruton *et al.*, 2009). There are few published life cycle assessments (LCAs) or techno-economic assessments for macroalgae biofuels (e.g. Alvarado-Morales *et al.,* 2013; Dave *et al.*, 2013; Langlois *et al.*, 2012).

Bruton *et al.* (2009) reviewed the potential of marine algae as a source of biofuel in Ireland, concluding that, although there is potential for energy generation, there are many obstacles to development and areas that require research. Marine algae are likely to make a modest energy contribution by 2020, and aquaculture is the most likely source (Bruton *et al.*, 2009). The development of commercial-scale biogas production from macroalgae seems likely to be best realised where a suitable coastline is available, in island communities.

1.5 A case study in marine bioenergy: the Isle of Man

The Isle of Man (IoM) is located in the Irish Sea (between Ireland, Scotland, England and Wales) and has population of approximately 80,000 people. The island imports gas and exports electricity (to the United Kingdom). Further to a recent report (commissioned by the IoM government) on renewable energy options, work at Durham University has considered various factors relating to the potential of macroalgae as a source of renewable energy and business for the IoM. Prior to this, there were no studies on the use of AD in the IoM, although a publication by Cleantech Investor Ltd. (2012) focused on offshore renewable energy.

Due partly to its attractive taxation status and opportunities for investment, the IoM considers itself well-placed for development of business in renewable energy. In addition, its landmass is relatively small, its coastline extensive and its community structure mostly coastal. The territorial seabed owned by the IoM extends 12 nautical miles (22.22 km) outward. Coastal areas could be suitable for the cultivation of macroalgae, and Douglas Borough Council (Douglas being the capital) already pays for the disposal of beach-cast macroalgae that could potentially be used in bioenergy production. In summary, the IoM provides a good case study in marine bioenergy. This will provide data that can benchmark an assessment of the potential for macroalgal growth in coastal waters around the United Kingdom to supply gas to the local domestic gas market. The only major-scale trial on AD of macroalgae (in Matsui *et al.,* 2006) will form a key reference in the study.

As part of the project at Durham University, potential growth rates of cultivated macroalgae will be assessed on a small scale in four key zones in coastal waters around the IoM (H.C. Greenwell, pers. comms, 2013). Available volumes of beach-cast will also be assessed, and the total yield of macroalgae thus estimated (H.C. Greenwell, pers. comms, 2013). Stakeholder perceptions and public acceptability will be studied through socio- and technological assessments relating to biogas technology, undertaken by Durham University Business School and Willow Research (H.C. Greenwell and E.F. Greenwell, pers. comms, 2014). Work has been done on thermochemical processing of macroalgae (e.g. Rowbotham *et al.*, 2012; Rowbotham *et al.*, 2013). In contrast to this, experimental work in the current thesis will focus on the potential for anaerobic co-digestion of macroalgae and selected wastes, outlined in section 1.6.

1.6 Aims and objectives

The present project aims to extend the feasibility study of macroalgae biogas for the Isle of Man, introduced in section 1.5. The largest uncertainty thus far concerns the effect of anaerobic co-digestion of macroalgae and waste materials from the island.

This thesis seeks to address the following specific objectives:

A. *Experimental assessment of co-digestion of macroalgae with waste streams*
   Laboratory-scale anaerobic digestion trials will be undertaken. Macroalgae (species *Laminaria digitata)* from UK coastal waters will be used as an individual feedstock, and in co-digestion with selected wastes (creamery waste and sewage pellets) from the Isle of Man. Results will be compared with those from similar trials undertaken elsewhere, and implications for larger-scale production considered.

B. *Assessment of the potential impact of a macroalgae anaerobic digester on the Isle of Man*
   Using data from the above laboratory trials, an assessment will be made of the implications for treating macroalgae and waste streams (creamery waste and sewage pellets) using an anaerobic digester on the Isle of Man.

1.7 Hypotheses

The following null hypotheses are made:

- *Null hypothesis 1*

  The addition of macroalgae to inoculum will have no effect on the volume of biogas produced in anaerobic digestion batch trials, as compared with the volume of biogas produced from inoculum only.

- *Null hypothesis 2*

  The addition of macroalgae plus sewage sludge pellets to inoculum will have no effect on the volume of biogas produced in anaerobic digestion batch trials, as compared with the volume of biogas produced from macroalgae added to inoculum.

- *Null hypothesis 3*

  The addition of macroalgae plus creamery waste to inoculum will have no effect on the volume of biogas produced in anaerobic digestion batch trials, as compared with the volume of biogas produced from inoculum only.

- *Null hypothesis 4*

  The addition of creamery waste plus macroalgae to inoculum will have no effect on the volume of biogas produced in anaerobic digestion batch trials, as compared with the volume of biogas produced from macroalgae added to inoculum.

- *Null hypothesis 5*

  An increase in the ratio of creamery waste to inoculum will have no effect on the volume of biogas produced in anaerobic digestion batch trials, when the same mass of macroalgae is added to this mixture of creamery waste and inoculum.

- *Null hypothesis 6*

  An increase in the mass of macroalgae added to a mixture of creamery waste and inoculum will have no effect on the volume of biogas produced in anaerobic digestion batch trials.

Part of the rationale is that the feedstocks will differ in composition, which is expected to affect digestion and biogas production. Adjustment of solids (total and volatile) is also expected to have an effect. Differences in organic loading may increase biogas yield due to more material being available for digestion, or may inhibit methanogenesis due to overloading.

2 METHODOLOGY

2.1 Anaerobic digester feedstocks

2.1.1 Macroalgae

Species selection

The macroalgae species *Laminaria digitata* (Hudson, Lamouroux) was selected as relevant to the current study due partly to its abundance in coastal waters around the IoM and the UK. In addition, although composition varies with season etc., the species has good potential for AD due to high carbohydrate content (e.g. Adams *et al.*, 2011b). *Laminaria digitata (L. digitata)* is a brown macroalgae (kelp) that is classified as shown in table 2.1.

Phylum	Ochrophyta
Class	Phaeophyceae
Order	Laminariales
Family	Laminariaceae
Genus	*Laminaria*

Table 2.1 Biological classification of the macroalgae species *Laminaria digitata*

Description and collection

Laminaria digitata was collected in a previous study (Redden, 2013). The species can be identified by its blade, which is long, broad, flat and dark brown. Depending on its age and the prevailing water movement, this blade is frequently divided into long 'digits'. *L. digitata* has a smooth, flexible stipe that has an oval cross-section. The species grows attached, reaching around 2-4m in length, and is found in lower littoral to sub-tidal zones. Whilst it prefers water movement, it may be found growing in sheltered as well as very exposed areas and in weak to very strong (> 6 knot) currents.

The site of collection was a 1 km area of Boulmer Beach in Northumberland, UK, centred on NU 267 137. Samples were taken monthly, from July 2009 to June 2011 inclusive, on days corresponding as closely to the lowest (spring) tide of the month as was feasible (table 2.2). In the present study, several of these monthly samples were selected and subsampled as outlined below.

Subsampling and preparation

Subsamples were taken from selected *Laminaria digitata* samples previously collected by Redden (2013) as described in above. The samples were stored in various freezers (-18 to -20 °C, and -80 °C) before being used in the present study.

As described by Redden (2013), the macroalgae was rinsed with seawater (pumped into the Dove Marine Laboratory, Newcastle University, from Cullercoats Bay, Northumberland, during mid-tide), in order to remove mud, sand and attached epiphytes from the macroalgae. Following this, the samples were stored in the dark at 4 °C. Within 48 hours, they were rinsed in tap water (at least four times their volume) in order to detach epifauna. Surface water was then removed by leaving the macroalgae to drip-dry for 20 minutes, before spinning it in a salad spinner (OXO Good Grips) for 1 minute. The material was then chopped to approximately 2 cm^2 and mixed.

The composition of a single macroalgae species varies seasonally (e.g. Black, 1950) and it was decided that some of this variation would be incorporated in the present study. Due to lack of material from some of the months of sampling, it was not possible to mix samples from 12 consecutive months without using the older and less fresh material. Quarters of a 12-month period were thought sufficiently representative. August 2010, November 2010, February 2011 and May 2011 were selected as the four most recent months that were equally spaced and had material remaining (table 2.2). An additional factor to consider in selecting quarterly samples could have been growth and sugar content of the macroalgae in relation to season. The growth rate of *Laminaria digitata* is seasonal, increasing from February to July and declining from August to January (Hill, 2008a). However, in-depth consideration of seasonal changes was beyond the scope of the present study. Composition will differ between years and locations, as well as due to other factors (e.g. reviewed by Redden, 2013) and the months selected were thought appropriate for the purpose.

A subsample of 131.76-225.49 g was weighed out of each of these four frozen (-18 to -20 °C) macroalgae samples using an analytical balance (Mettler Toledo, NewClassic, ML4002/01). The material had previously been chopped to approximately 2 cm^2, but was chopped slightly more using scissors. Only a small amount of defrosting occurred during this preparation, and degradation was minimal. After preparation, the subsamples were returned to the freezer (-18 to -20 °C).

Year	Month	Day	Time	LW (m)		Year	Month	Day	Time	LW (m)
1	Jul-09	23	11:24	0.21		2	Jul-10	14	12:22	0.22
1	Aug-09	20	09:20	0.11		2	Aug-10	12	12:04	0.02
1	Sep-09	19	10:43	0.21		2	Sep-10	9	10:57	0.06
1	Oct-09	19	09:56	0.52		2	Oct-10	7	09:49	0.33
1	Nov-09	16	08:52	0.95		2	Nov-10	5	08:25	0.62
1	Dec-09	16	09:12	1.36		2	Dec-10	9	11:31	1.42
1	Jan-10	15	09:36	1.43		2	Jan-11	21	10:27	0.89
1	Feb-10	16	10:48	1.07		2	Feb-11	19	10:09	0.67
1	Mar-10	17	10:23	0.94		2	Mar-11	21	10:27	0.43
1	Apr-10	15	09:57	0.91		2	Apr-11	19	10:08	0.43
1	May-10	14	09:33	0.94		2	May-11	19	10:40	0.51
1	Jun-10	16	13:21	0.52		2	Jun-11	17	10:31	0.60

Table 2.2 Schedule for sampling of macroalgae, with corresponding time and height of low water (LW), 2009-2011 (Redden, 2013). LW indicates the height of low water at Tynemouth, Northumberland. (Time and LW reproduced from Port of Tyne Authority Tide Tables). Grey shading indicates the samples used in the present study

The frozen subsamples were later transferred to a freezer at -80 °C, before being individually freeze-dried (using a Christ Alpha 1-4 LSC with a shelf temperature of – 20 °C and a pressure of 1.810 mbar). Although macroalgae would not be dried for large-scale AD, drying was thought suitable for laboratory purposes. It enabled the material to be more easily separated and weighed in equal amounts than when wet, and had the additional benefit of standardising the macroalgae used in the digestion trials. Freeze-drying was preferred over oven-drying as the latter was likely to degrade the samples.

Using the mass of each of the four subsamples before and after freeze-drying (recorded to the nearest 0.01 g), mean lyophilised mass was calculated as % dry mass. After being freeze-dried and weighed, each subsample was homogenised using a coffee grinder (almost identical to James Martin ZX809X, by Wahl). 15 g (to the nearest 0.05 g) of each was then weighed using a 4 decimal place (d.p.) micro-balance and transferred to a jar. The jar was shaken thoroughly by

hand in order to mix the material, and then wrapped in foil to minimise degradation in light. The macroalgae was kept in a desiccator at room temperature when not needed. Freeze-drying eliminated the need for cold storage (Holm-Hansen, 1973). Some of the freeze-dried, ground material is shown in figure 2.1.

Figure 2.1 Photograph of freeze-dried, ground macroalgae (*Laminaria digitata*) used in anaerobic digestion trials

Measurement of solids

The lyophilised, ground macroalgae used in the AD trials were tested for solids using standard methods for the examination of water and wastewater (Clesceri *et al.* 1998). The methods used were appropriate for solids in sewage. The same method was used for macroalgae as for creamery waste (section 2.1.3) and digester samples (section 2.2.9), but using solid material in crucibles instead of liquid in filtration. However, solids were reported in mg/kg, as total solids (TS) and volatile total solids (VTS) (as in methods intended for sludge, Clesceri *et al.*, 1998), rather than as suspended solids (SS) and volatile suspended solids (SS).

Three macroalgae subsamples of 1 g (to the nearest 0.1 g) each were weighed into a dried crucible. The samples and crucibles were put into an oven at 104 °C for 15 min before being cooled in a desiccator and weighed to the nearest 0.1 mg using an analytical balance. The crucibles and contents from were then put into a muffle furnace at 550 °C for 10 min, cooled in a desiccator and reweighed to the nearest 0.1 mg.

Elemental analysis

Three subsamples of lyophilised, ground macroalgae were analysed for percentage carbon, hydrogen and nitrogen (% CHN, determined to 2 d.p.). They were weighed using a 7-place microbalance and analysed in tin capsules with nickel sleeves. The elemental analyser was an

Exeter Analytical CE440 with a horizontal furnace. The combustion furnace temperature was 975 °C. The reduction tube temperature was 620 °C. The carrier gas was helium. Thermal conductivity detection was used. Calibration was with acetanilide (batch 183632 from Exeter Analytical).

2.1.2 Sewage pellets

Description and collection

Pellets of sewage sludge (approximately 150 g) were provided by the Isle of Man Water and Sewerage Authority in December 2013. Figure 2.2 shows some of the sample (before grinding as described below. The pellets are formed on the island, at Meary Veg sewage treatment plant, from sludge dried at over 400 °C. Approximately 1000 tonnes of pellets are produced each year. They have previously been used as a soil conditioner on farmland, but are currently disposed of at the energy-from-waste (EfW) facility (operated by SITA), their most economical disposal route on the island. Typical composition as determined in previous analyses at Meary Veg is shown in section 3.1.2.

Figure 2.2 Photograph of sewage sludge pellets used in anaerobic digestion trials. (Pellets are shown whole but were ground for trials)

Subsampling and preparation

The sample of pellets provided was stored in containers at room temperature for around seven months. Most of the pellets (one container with 201.0643g and another with 191.5347g) were then weighed before being freeze-dried (at -100 °C and approximately 0.050 atm, using a Christ Alpha 1-4 LDC-1m). They were then re-weighed.

Using the mass of each of the subsamples before and after freeze-drying (recorded to the nearest 0.1 mg), mean lyophilised mass was calculated as % dry mass. However, weighing of the pellets was particularly problematic due to their highly hygroscopic nature.

A subsample of approximately 16 g was then homogenised using a coffee grinder (almost identical to James Martin ZX809X spice and coffee grinder, by Wahl) and this ground material was stored in a desiccator at room temperature until needed.

Measurement of solids

The lyophilised, ground sewage pellets used in the AD trials were tested using the same methods as described in section 2.1.1.

Elemental analysis

Three subsamples of lyophilised, ground sewage pellets were analysed for percentage carbon, hydrogen and nitrogen (%CHN, determined to 2d.p). The elemental analyser, procedure and parameters were as described for macroalgae in section 2.1.1.

Additional analyses (secondary results)

Chemical analyses were undertaken for a report (by Direct Laboratory Services) on a 2005 sample of sludge pellets from the IoM. The composition of the pellets used in the current study is expected to differ little from this earlier sample. The total of each of the following elements as mg/kg was determined by inductively coupled plasma (ICP) analysis: aluminium, iron, phosphorous, potassium, sulphur, magnesium, lead, nickel, zinc, cadmium, chromium and copper. The methods used to determine pH, oven dry matter, total nitrogen (Kjeldahl), total mercury and ammonium-N are not noted here.

2.1.3 Creamery waste

Description and collection

Creamery waste was provided by the Isle of Man Creamery Ltd. The creamery forecasts that 22 million litres of milk will be processed in 2015. Waste comes from two streams. The first is pre-wash (mainly tank washings with a low content of milk solids). The second waste stream is de-fatted whey from the process of cheese-making. To the best of the creamery's knowledge, all wastes are of an organic nature and contain no harmful biological agents. Protein in the milk received peaks around October or November and has a low point around February or March.

Current average figures for fat, lactic acid, solids and pH (provided from measurements by workers at the creamery) of waste discharged are given in section 3.1.3.

The wastes are discharged into the sea, under licence from the IoM government. The current licence expires in October 2019. From Mondays to Fridays, the factory operates from 0:00 to 17:00. At weekends, it operates from 08:00 to 16:00. Wash waters are produced intermittently throughout these timeframes. At present, cheese is made on Mondays, Wednesdays and Fridays. Approximately 18 vats of cheese are produced per week.

Approximately 8L of waste was sampled and collected within the same day in January 2014. The sample was said to be a typical wash, consisting of wash water from the cleaning of production equipment, plus defatted whey from the cheese-making facility. However, as only two vats of cheese were made that day, the sample was said to be mostly wash water and only possibly contained whey. The waste was transported from the creamery in cool packaging and then refrigerated from the same afternoon until the next morning. It was then transported in cool packaging before being frozen at -18 to -20 °C approximately 24 h after collection.

Sub-sampling and preparation

The creamery waste remained in the freezer (-18 to -20 °C) for approximately six months before being defrosted over a period of less than one week at approximately 4 °C.

Figure 2.3 Photograph of the vial on a stick used to sample creamery waste for testing of solids and pH

Once defrosted, the waste was decanted into one container and then shaken vigorously by hand for 30 seconds to mix. The lid was then quickly removed and approximately 14 ml of waste sampled through the column, as soon as possible, using a vial attached to a stick (figure 2.3). This subsample was transferred to a centrifuge tube and frozen for later testing of solids and

pH, described below. The process of mixing and sampling was repeated in order to obtain five replicates in total. The remainder of the waste was shaken by hand and an amount then quickly poured into a smaller container. This process was repeated (with shaking each time before pouring) until the waste had been divided between several containers. The aliquots were then frozen at -18 to -20 °C. They were assumed to be homogenous, and one was defrosted at approximately 4 °C, over a period of several days, as needed for each digestion trial. It was assumed that the waste did not undergo decomposition whilst frozen. Some of the defrosted creamery waste is shown in figure 2.4.

Figure 2.4 Photograph of defrosted creamery waste used in anaerobic digestion trials

Measurement of pH

The five replicates of creamery waste sampled (as above) were shaken by hand and then tested using pH test strips (pH-Fix 0-14, Fisherbrand®). These replicates had previously been tested for solids (as below) but had sufficient waste remaining.

Measurement of solids

AD trials would usually be based on solids content. However, as the creamery waste contained few solids, its use on a solids basis would have required addition of large volumes. It was instead used on the basis of volume (mixed with the inoculum as described in section 2.2.3) and suspended solids (SS) and volatile suspended solids (VSS) were tested. Five replicates of creamery waste (sampled as described above) were tested using standard methods for the examination of water and wastewater (Clesceri *et al.*, 1998). 10 ml of each sample was filtered.

Each aliquot was filtered through a prepared glass microfiber filter paper (GF/A, Whatman). The sample and paper were dried at 104 °C for one hour, cooled in a desiccator and weighed to the nearest 0.1 mg. SS were then reported as mg/L.

The papers and contents from the above were ignited at 550 °C for 15 min, cooled in a desiccator and weighed to the nearest 0.1 mg using an analytical balance. VSS were then reported as % in SS.

As the creamery waste had visibly low solids content than the inoculum, a larger volume was filtered than for the digester samples (tested as described in section 2.2.9).

2.2 Batch anaerobic digestion trials

Laboratory-scale AD assays can be carried out in batch- or continuous mode. The batch test is the more widely used, being less laborious, and its principle is the measurement of biogas or methane production. However, a review by Raposo *et al.* (2011) showed a lack of uniformity in data reported from anaerobic batch tests (probably due to the use of different inocula and experimental conditions) and suggested a need for comparability of tests carried out in different laboratories.

2.2.1 Digester set-up

The set-up of the anaerobic digesters is shown in figure 2.5. One type of tubing (Versilic® silicone tubing, 1.5mm inner diameter, 3.0 mm outer diameter, Universal Biologicals Ltd.) was used throughout. When first set up, the water in the cylinders was clear and the tubing was free of liquid. Purple colouring was caused later, by potassium permanganate mixed with water, when backflow issues occurred in trial 2 (see section 3.3.6) and trial 3 (see section 3.4.6).

Each digester consisted of a 250ml bottle that had a cap with two ports and various fittings (Duran®). A blind cap was added to the unused port on each lid. The other port was fitted with a hose connection that had an insert for tubing. Tubing was fed through, leaving the end as far up into the headspace of the hose connection as was practical. This tubing led to a three-way Luer lock stopcock (Sigma-Aldrich) that was designed for liquid chromatography and could be switched to allow sampling of biogas.

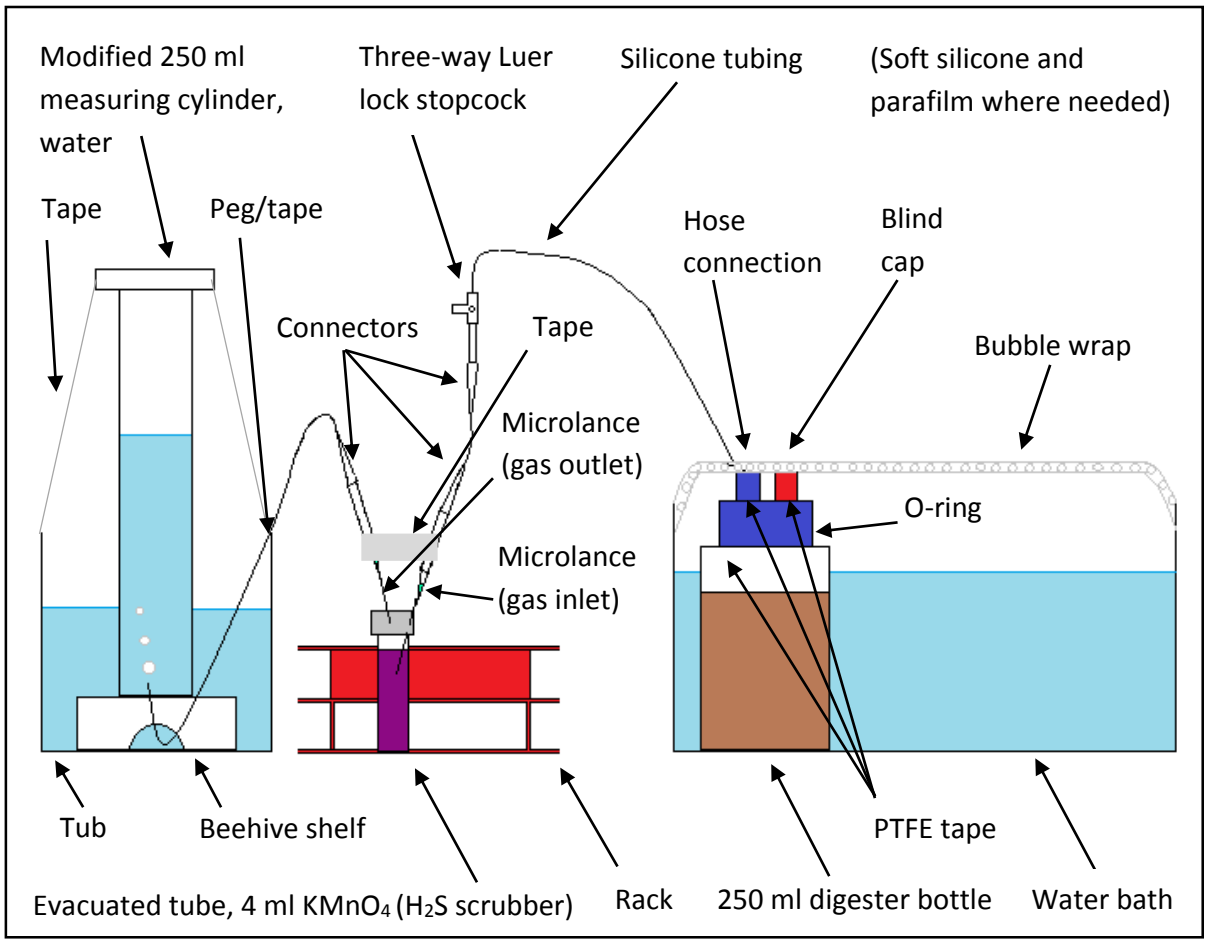

Figure 2.5 Diagram of the anaerobic digester set-up (not to scale)

Although only trace amounts (1%) are typical in gas from anaerobic digesters (Polprasert, 1996), H_2S is a corrosive, toxic and noxious gas. After each stopcock was an H_2S scrubber made from a 3 ml evacuated tube (BD Vacutainer®) into which two microlances (BD Microlance™) had been inserted. Each lance was connected to tubing using part of a Luer lock syringe and a pipette tip. One lance was positioned above the other, with minimal headspace, forming a gas outlet. The lower lance formed a gas inlet and the two were secured together with tape. Before each trial, a fresh 4 ml of potassium permanganate ($KMnO_4$) solution (volumetric, 0.2 M Sigma Aldrich) was added to each scrubber. The biogas subsequently produced by digestion was bubbled through this $KMnO_4$ so that any H_2S contained would be oxidised and effectively removed. The inoculum was not thought to be of concern in terms of H_2S. Based on comparable results for typical sulphur (S) content of beach-cast macroalgae, the approximate S content of the macroalgae used was assumed to be 1% weight (e.g. Adams *et al.*, 2011b). Given the estimated volume (up to approximately 0.28 L/g/day, Redden, 2013) of biogas that would be produced, and the moles of H_2S generated assuming 100% conversion of S, it was assumed that neither total throughput nor concentration at any given time would prevent complete

scrubbing of the H_2S by 4 ml $KMnO_4$ per trial. Fresh permanganate was put into the scrubbers before each subsequent trial.

The tubing from the gas outlet in each scrubber led to a modified measuring cylinder used for gas collection as described in section 2.2.10. (In the first trial, a second three-way stopcock was positioned after the scrubber. The intention was to sample gas from after, as well as before, the scrubber. However, issues with suction of water from the cylinders prevented post-scrubber sampling, so subsequent trials had only one, pre-scrubber stopcock). Each cylinder (250 ml) had been cut and had a rim added, allowing it to stand upside-down on a beehive shelf (from Philip Harris). A beehive shelf (figure 2.6) is a type of stand designed to support a jar or tube used in a pneumatic trough for gas collection. Each shelf was placed inside a 2300 ml plastic tub (with a sufficiently flat base) to which approximately 1500 ml deionised (DI) water was added. Tubing was fed through the beehive shelf and pegged to the side of the tub. It was later taped partway through a trial after it was noted that, despite having being tested with a syringe before the trial, some of the pegged tubing was blocking gas passing into the cylinders.

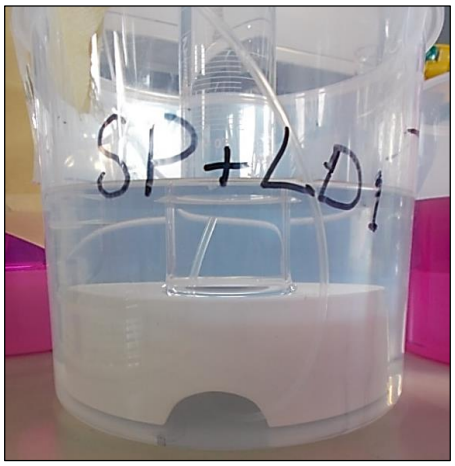

Figure 2.6 Photograph of a beehive shelf used to collect biogas in a cylinder

Each cylinder was filled to approximately 250 ml with DI water, covered and inverted before being quickly placed on the beehive shelf with the end of the tubing inside. As much as possible of the initial water was retained in the cylinder, but any loss was accounted for by calibration (section 2.2.10). Each cylinder was secured with tape in order to stand upright and as level as possible. 5 ml of hypochlorite was then added to each tub in order to discourage the growth of mould. Several ml of air were injected through each stopcock and into the cylinders, to ensure that there were no blockages and to minimise water held inside the tubing. This set-up

eliminated the potential problems of sampling gas from the cylinders, where the gas might have dissolved into the liquid.

In order to improve gas-tightness, PTFE tape (polytetrafluoroethylene, thread seal tape) was wrapped around the rims and ports of the digester bottles. Soft silicone and parafilm were added to the bottle ports, scrubbers and tubing connectors where needed. An O-ring (gasket) was inserted into each bottle cap. Before each digestion trial, the assembled bottles, scrubbers and connectors were immersed in water and tested by being provided with a constant flow of compressed air. Adjustments were made as needed, until a lack of bubbles confirmed gas-tightness. It was assumed that the scrubbers remained gas-tight after the tube caps had been removed and replaced to allow addition of permanganate, as the bungs inside should not have been affected.

2.2.2 Inoculum

The initial inoculum was sludge mixture known to contain anaerobic bacteria. This was obtained from the primary and secondary treatment stages of a working anaerobic digester at Bran Sands sewage treatment works (STW, Northumbrian Water Ltd.). Industrial wastewaters are treated in the activated sludge plants, with this degradation of organic matter producing the sludge due to growth of bacteria in the aeration lanes.

The sludges digested at Bran Sands can originate from anywhere in the Northumbrian Water catchment. They are imported either in a cake (24% dry solids) or in a slurry (4% dry solids). All sludges comprise raw solids, solids settled from raw sewage and non-biologically treated and surplus activated sludge (SAS). The indigenous sludge from the Bran Sands raw sludge and SAS aerobic treatment plant can make up 50% of all the solids in the feed to the digesters.

Following delivery, the sludge sample was shaken by hand to mix before being divided between five containers. These were stored in a cold room at approximately 4 °C. A post-centrifuge sample would have much lower solids content than one taken before centrifuging, but would contain a polymer. The sludge used as inoculum was a pre-centrifuge sample of approximately 12 L. Most of the volatile solids in a sample from the primary stream should already have already been removed, provided the AD was efficient. Testing (section 2.2.9) showed high solids content. The majority of the solids could have been removed from the pre-centrifuge sludge by leaving it to settle, although it would still have remained quite ‘dirty’. Alternatively,

the sample could have been filtered or diluted. However, given time constraints, it was decided that solids content would not be adjusted (except where creamery waste was added as described in section 2.2.3) as this might have caused a lag phase in the digestion (Mata-Alvarez *et al.*, 2000).

2.2.3 Experimental design for batch digestion trials

Batch trials were carried out using macroalgae as an individual feedstock, and in co-digestion with creamery waste or with sewage pellets. Three trials were undertaken. Direct inter-trial comparison would be difficult due to unavoidable microbial changes in the inoculum during storage. However, each trial could be treated as a separate experiment, with fed digesters assessed relative to controls. It was thought simplest to include controls and repeats within the same trial, and change inputs between trials. Each control and fed digester type was triplicated. The experimental matrix is shown in table 2.3.

The null hypotheses are outlined in section 1.7. Given data from trial 1, the effect of adding macroalgae to inoculum could be assessed relative to a control (blank inoculum).

Data from trial 2 would enable the effect of adding macroalgae plus sewage sludge pellets to inoculum to be compared with the effect (as seen in trial 1) of adding macroalgae to inoculum. The effect of adding macroalgae plus creamery waste to inoculum could be assessed relative to blank inoculum. Additionally, the effect of adding macroalgae plus creamery waste to inoculum could be compared with the effect (as seen in trial 1) of adding macroalgae to inoculum.

Data from trial 3 would show the effect of increasing the ratio of creamery waste to inoculum in a mixture to which macroalgae is added. It would also show the effect of increasing the mass of macroalgae added to a mixture of creamery waste and inoculum.

For trial 1 and trial 2, 1 g/L (as lyophilised mass, section 2.1.1) was chosen as a reasonable loading that was thought unlikely to overload the digesters. In a similar study, digestion in feeding trials using this loading rate was found to be stable (Redden, 2013). In trial 3, a loading of 1 g/L was compared with 2 g/L.

	Control	**Fed digester type 1**	**Fed digester type 2**
Trial 1	250 ml inoculum (CONTROL 1) (CONTROL 2) (CONTROL 3)	250 ml inoculum 1 g/L *Laminaria digitata* (LD1) (LD2) (LD3)	N/A
Trial 2	250 ml inoculum (CONTROL 1) (CONTROL 2) (CONTROL 3)	250 ml inoculum 0.5 g/L sewage pellets 0.5 g/L *Laminaria digitata* (S+LD1) (S+LD2) (S+LD3)	125 ml inoculum 125 ml creamery waste 1 g/L *Laminaria digitata* (C+LD1) (C+LD2) (C+LD3)
Trial 3	4.46 ml inoculum 245.54 ml creamery waste (CONTROL 1) (CONTROL 2) (CONTROL 3)	4.46ml inoculum 245.54 ml creamery waste 1 g/l *Laminaria digitata* (C+LD.A1) (C+LD.A2 (C+LD.A3)	4.46 ml inoculum 245.54 ml creamery waste 2 g/L *Laminaria digitata* (C+LD.B1) (C+LD.B2) (C+LD.B3)

Table 2.3 Experimental matrix showing the input into each of the digesters in the three batch anaerobic digestion trials. (Digester names are bracketed)

The ratios of co-digestates in trial 2 were decided following results from trial 1. Those in trial 3 were decided following results from trial 2. The ratios of macroalgae to co-digestate used in the literature vary, but frequently the macroalgae forms less than 50% of the total feedstock (e.g. Briand and Morand, 1997, volatile solids basis; Cecchi *et al.*, 1996, total solids basis). If AD is to form a disposal route for beach-cast macroalgae on the IoM, the waste streams are likely to contribute least and the ratio in co-digestion would ideally be biased towards macroalgae. Following trial 1, it was decided that ratios of 1/1 in trial 2 would give scope for adjustment in either direction, in subsequent trials. This adjustment could be made on the basis of results from trial 2. A fourth trial was not undertaken in the time available, but it was thought that comparison of various co-digestate ratios could indicate whether the effect of co-digestion was additive or linear.

The creamery waste was mixed with inoculum and used on a volume basis due to low solids content (see section 2.1.3). In trial 2 the sewage pellets, like the macroalgae, were used on the basis of lyophilised mass (section 2.1.2). The 1 g/L was partitioned between the two co-digestates so that the loading was constant between trials 1 and 2.

In trial 3, based on relatively poor biogas production from sewage pellets in trial 2 (section 3.3.6), the pellets were discarded. The pellets had not yet been analysed for volatile solids (see section 3.1.2 for results), but were expected to have low VS content, having already undergone advanced oxidation. Additionally, as the licence for discharge of creamery waste expires in 2019, this is the principal waste in need of a disposal route. It would have been useful for experiments to indicate how much waste the creamery might be able to dispose of through AD. A possibility was to dilute the inoculum to different degrees, using creamery waste, and add macroalgae. However, macroalgae is of most interest as a feedstock on the island, and solids in the creamery waste will vary depending on production processes etc. (see section 2.1.3). Furthermore, the inoculum used in trials was high in solids and unrepresentative of the IoM, which would not have a sewage waste stream in its AD. 100% creamery waste could have been used. However, it was thought that this would give insufficient solids. In addition, the inoculum contained the methanogens necessary for starting the AD process within a reasonable time period. It was decided that in trial 3 two different loadings of macroalgae would be co-digested with creamery waste, plus a small amount of inoculum.

Results for control digesters in trial 1 (section 3.2.3) showed that the mass of VSS in the inoculum used in each digester was high relative to the lyophilised mass of macroalgae added. In deciding the ratio of creamery waste to inoculum, it was assumed that the creamery waste contained negligible solids. Solids contents in the range of 8.3-22% have been reported in macroalgae (Jard *et al.*, 2013b; Msuya and Neori, 2008; Lamare and Wing, 2001). For brown and red macroalgae, volatile solids contents are reported as ranging from 44.6-73.8% of dry solids (Jard *et al.,* 2013b). Given the high solids content of the inoculum, biasing the ratio of solids in the digester towards the macroalgae added (as lyophilised mass) would require the inoculum to be greatly diluted. (A loading of 1 g/L macroalgae in trial 3 was still needed for comparison with trials 1 and 2). 5 ml inoculum with 275 ml creamery waste (before 30 ml was sampled on day 1) was thought sufficient.

2.2.4 Initialisation of batch digestion trials

Before being used in trials, the digester bottles, caps, ports and inserts were soaked in hypochlorite solution (5% in deionised water) for at least one hour before being rinsed in tap- and deionised (DI) water. (For trial 1, the O-rings were not bleached but were rinsed with DI water. It was thought that any remaining bacteria had negligible effect on digestion as the inoculum was stored inside the bottles. Some inoculum was rinsed out of tubing for the first trial, after an initial attempt at digestion, when solids seemed to have been pushed up inside the bottles before sampling of aliquots. The tubing outlets were adjusted.

Although 'pre-culturing' (acclimation or adaptation) of the inoculum with the substrate is widely accepted, Raposo *et al.* (2011) note a lack of it being reported in biochemical methane potential (BMP) tests, which fit well with use of non-acclimated inocula. Given time constraints and the fact that batch- rather than feeding trials were undertaken, the inoculum was not pre-cultured. Feedstocks were added as described in section 2.2.5.

Despite having been shaken, when the inoculum was divided between five containers (section 2.2.2) the final sample was most viscous. For each trial, in order to improve homogeneity, an amount was decanted from each of these containers and the sludge shaken by hand before being divided between the digester bottles. The necessary amount of sludge (see section 2.2.3) was added to each bottle, and the remainder returned to the cold room (approximately 4 °C). Approximately 30 ml of inoculum was spilled from the LD3 digester at the beginning of trial 1 (as deduced from end-volume). Macroalgae had been added and aliquots were being sampled.

The inoculum was degassed during storage, but was not completely degassed (for example, by incubation) at the start of trials.

After each trial, the inoculum used in the digesters was disposed of. For each subsequent trial, inoculum was mixed from the remainder of the original sample and divided between the bottles as described above.

2.2.5 Operational conditions of batch digestion trials

Temperature

The water bath (Fisher Scientific) was filled so that the water reached the shoulders of the bottles, and was covered with bubble wrap for insulation (see figure 2.7). Evaporation occurred, so the water was topped up daily. The bubble wrap was removed for several days during part of one trial. However, this seemed to have negligible effect on the temperature of the water bath. Several ml of hypochlorite or Dettol were added to the water for each trial, to reduce fungal and bacterial growth. Throughout each trial, the temperature of the water bath was held at 35°C (i.e. within the mesophilic range of 20-45 °C) and the temperature (single-point) recorded daily using a mercury thermometer. Readings for day 4 and day 15 in trial 1 and days 20-22 in trial 2 were missing from the dataset, but no unusual readings were noted.

Figure 2.7 Photograph of the anaerobic digesters insulated in the water bath

Testing of methanogens

At the start of each trial, 0.5 g/L of sodium acetate (powder, bioreagent, Sigma Aldrich) was added to each digester bottle. 0.14 g was to the nearest 0.01 g using a 4 d.p. balance. The bottles, with blank caps, were then shaken. The ported caps were then replaced quickly and the bottles placed in the water bath. If present, acetoclastic archaea will use sodium acetate as a resource, causing a rapid increase in methane gas production (indicated by gas bubble

formation) within 5 minutes of the sodium acetate being added. Trial 1 had the longest interval between addition of acetate and feedstocks. In the initial attempt at this trial, there were issues with blockage of tubing and gas-tightness of the set-up following addition of acetate. The trial was paused and the bottles kept in the cold room (around 4°C) while the set-up was modified. Macroalgae was added once problems were resolved. In trial 2, feedstocks were added sooner after acetate. As backflow occurred near the start of trial 2, it was thought that the digesters might have used the resource quickly and needed further feeding. In trial 3, the interval after adding acetate was shortened, but backflow still occurred soon afterwards.

Anaerobic headspace

The headspaces of the digester bottles were not flushed (e.g. with air or nitrogen), partly due to impracticality given the set-up, and partly because this would add to costs on a commercial scale. Given the small amount of headspace, the digesters were assumed to be sufficiently anaerobic and it was thought that any air would be pushed out as biogas was released.

Addition of feedstocks

After sodium acetate had been added to test each bottle (see above), the bottles were removed from the water bath and substrates added (day 1). The substrates had been weighed to the nearest 0.01 g using a 4 d.p. balance. The loadings are shown in the experimental matrix in section 2.2.3.

Macroalgae and sewage pellets, where used, were added on the basis of lyophilised mass (section 2.1.1 and section 2.1.2 respectively). Where creamery waste was used, it was mixed with the inoculum (on the basis of volume) immediately before addition of sodium acetate or macroalgae. Following addition of all feedstocks, the digesters were sampled as described in section 2.2.6.

Retention time

Each trial was carried out over a period of 21 days (days 1-22). After feedstocks were added on day 1 (section 2.2.5), the digesters were left without further feeding. A wide range of incubation times are reported in the literature, but most of the material would be expected to digest within 14 days (e.g. H. Redden, pers. comms, 2014). Shi *et al.* (2013) suggest that, kinetically, biodegradation of macroalgae is largely completed in around 20 days. A 21-day period was

chosen as sufficiently long to give reasonable results, yet short enough to allow several trials to be carried out.

Agitation

Following addition of feedstocks (as above), blank caps were added to the bottles, which were agitated and sampled as described in section 2.2.6. The blank caps were then replaced with ported caps, and the bottles returned to the water bath (as above).

During trial 1, the digesters were agitated once daily for days 1 to 3 inclusive. Due to initial concerns about blocking the gas outlet with inoculum, each digester was given a blank cap and shaken by inversion for before the ported cap was replaced. The digesters were left uncapped for minimal time while caps were exchanged. However, it is thought that while the ported caps were off, the directions of the three-way stopcocks were not adjusted, so any biogas in the tubing was left open to the air. Regardless of the stopcock switch positions, removal of the caps is likely still to have caused some biogas loss or mixing with air because the length of tubing between each cap and stopcock was not clamped. The methodology was adjusted and the trial 1 bottles were not agitated from day 4 onwards. Shaking sideways was suggested as a better method of agitation than inverting with blank caps, as this would prevent gas loss when caps were swapped. However, it was thought best not to agitate at all as the digesters were in batch mode rather than being fed at intervals. The gas was expected to work its way out of the digesters eventually, despite lack of agitation.

At the outset of trial 2, it was planned that the digesters would not be agitated. However, issues with backflow occurred early in the trial, affecting cylinder readings. After sodium acetate had been added for testing methanogens, it was left to digest overnight. By the next day, suction of potassium permanganate out of, and water towards, the scrubbers was observed. The digesters were given blank caps and stored temporarily at around 4°C while the scrubbers were flushed and refilled.

Feedstocks were added, digesters sampled and the trial started the day after this backflow was noted. There were subsequently further issues with backflow. Gas bubbles could be seen forming, but were not passing any further than the water that had been sucked to above the scrubbers. The set-up had not been changed between trial 1 and trial 2, except in trial 2 there

was only one stopcock for gas-sampling after each scrubber and none before. (It was found in trial 1 that suction of liquid prevented sampling from the stopcocks before the scrubbers).

It was noted that raising one of the cylinders and putting it back down helped to move liquid back towards the cylinder. Some alteration of gradients and tubing bends might have helped to remediate backflow. Slight adjustment was tested when backflow issues began. Propping the stopcocks on top of cylinders generally helped. However, there was little that could be done without drastically altering the set-up mid-trial. It was decided to let trial 2 run for approximately a week and see whether problems were resolved without intervention. One-way valves could have been used if suction of water and permanganate into the digesters seemed likely. Sampling of gas following cylinder readings on day 3 caused further suction of water and permanganate, with permanganate moving close to one of the control digesters. This drained back away from the digester when the stopcock (with tubing) was propped on top of a cylinder.

Agitation was expected to help to displace total gas and, provided it was consistent, should allow day-by-day comparison. The digesters were agitated once daily (after cylinder readings) from day 6 to day 21 inclusive. Although this altered the parameters of the experiment and affected the rate curves, it was thought best that the trial provided some data on biogas volumes. The agitation seemed to remediate backflow and data output improved, so it was decided that one-way valves would be unnecessary. In order to prevent any gas loss as occurred when blank caps were used in trial 1, the digesters in trial 2 were left with the ported caps attached to tubing, and shaken sideways by hand for 20 s.

In trial 3, the digesters were agitated by hand once daily, after cylinder readings. Based on observations from trial 2, it was thought that this agitation would prevent backflow and that one-way valves would be unnecessary. Each digester in trial 3 was shaken sideways for 20 s as in trial 2. Backflow issues occurred from day 3 onwards, but agitation was continued as on the previous days.

2.2.6 Sampling of digester bottles

On day 1 and day 22 of each trial, each bottle was shaken by inversion for 20 s. Three aliquots of 10 ml (or one aliquot of 30 ml, divided later) were then poured from each bottle.

2.2.7 Measurement of pH in digester bottles

Most batch tests are carried out between pH 7 and 7.8, and the pH adjusted if necessary. The pH was not controlled in the present study. For day 1 and day 22, the pH of each anaerobic digester bottle was tested in triplicate, using the samples collected as described in section 2.2.6. Approximately 2 ml of each 10 ml aliquot (balanced by mass) was pipetted into a microcentrifuge tube and then centrifuged for 10 min. For each sample, several drops of supernatant (at around room temperature) were transferred to a pH test strip (pH-Fix 0-14, Fisherbrand®) and the pH recorded.

2.2.8 Measurement of salinity in digester bottles

The 10 ml aliquots removed, prepared and tested for pH as described in section 2.2.6 and section 2.2.7 were also used to test the salinity of each bottle in triplicate, for day 1 and day 22. The salinity of the remaining supernatant was tested using a refractometer (Bellingham and Stanley, E-line refractometer).

2.2.9 Measurement of solids in digester bottles

The 10 ml aliquots (30 ml per bottle) removed (as described in section 2.2.6) were tested for suspended solids (SS) and volatile suspended solids (VSS). A small amount (approx. 2 ml) had first been removed to test pH (section 2.2.7) and salinity (section 2.2.8). The remainder was tested using standard methods for the examination of water and wastewater (Clesceri *et al.* 1998), as described for creamery waste in section 2.1.3. Due to loss of material, measurements for some aliquots were substituted with an additional analysis on another aliquot from the same digester.

2.2.10 Measurement of biogas production

The set-up of the cylinders used for collection of biogas is described in section 2.2.1. Before the start of each trial, a reading was taken from each cylinder. Once the trial had begun, the volume of gas produced was monitored by taking a daily reading from each cylinder throughout the 21-day period, and correcting for gas sampled as described in section 2.2.11. It was noted during trial 1 in particular that small amounts of gas tended to collect in the scrubbers. The racks containing the scrubbers were tapped several times before daily readings. This generally produced a few bubbles and then had no further effect. It had no effect at all at some points or in some trials, and was not always done. Because the total volume of gas produced by some digesters exceeded the readable capacity of the corresponding cylinders, some cylinders

required recalibration during a trial. In this case, the stopcocks were closed to prevent biogas escaping, and the cylinders removed and then refilled as above. Once the cylinders had been inverted and secured, the date was noted and new readings were taken. The stopcocks were then reopened and collection and monitoring of gas continued. As atmospheric pressure was expected to affect the water level in the cylinders, mean sea-level pressure (MSLP, Met Office) in the area at the time of cylinder readings was noted daily. Where readings overlapped adjacent hours with different pressures, the mean of the two pressures MSLP values was taken. In the first trial, an additional (control) cylinder was set up, unconnected to a digester.

As the intervals between daily cylinder readings ranged from 21 to 26 hours, and were not always 24 hours, the rate of gas production between readings was calculated on a 24-hour basis, using the time (to the nearest hour) since the previous reading.

2.2.11 Sampling of biogas

Biogas was sampled from each digester approximately every fourth day. Samples were taken on days 3, 7, 10, 13 and 16 in trial 1, and on days 3, 7, 10 and 13 in trial 2. (No gas was sampled in trial 3, as sampling was expected to worsen the backflow of potassium permanganate and water already occurring. This backflow was more severe than in trial 2).

The number of samples was thought sufficient to be representative without being too excessive for gas chromatography (GC) analysis. It was not known before the trials what the activity levels of the digesters would be and the intervals were not spaced accordingly. Samples were not taken beyond day 16 in trial 1 because gas production seemed to have tailed off. The same pattern was followed in trial 2, but day 16 was omitted. Biogas was sampled from a three-way stopcock before an H_2S scrubber (section 2.2.41). Each time, a 3 ml sample was taken using a gas-tight Luer lock syringe (SGE) and injected into an evacuated tube (BD Vacutainer®). In some case, due to issues with moisture or suction of liquid in tubing, less than 3 ml gas was sampled, or a sample was taken but discarded, or a sample was not taken at all. (Overall biogas volumes were adjusted accordingly). In order to prevent degradation of the bungs in light, the tubes were wrapped in foil and stored in the dark until gas chromatography analysis (see section 2.1.12).

2.2.12 Gas chromatography and calculation of methane content

Gas chromatography (GC) was used to determine the methane content of the biogas sampled as described in section 2.2.11. The time between sampling and analysis was more than a month and would ideally have been shorter. Gas was sampled from each evacuated tube (section 2.2.11) using a 100 µl syringe and injected directly into a gas chromatograph (GC, Carlo Erba HRGC S160). All samples were analysed in triplicate (using three lots of 100 µl). The GC was fitted with a flame ionisation detector (FID) and HP-PLOTQ column (0.32 mm diameter, 30 m length and 20 µm film; Agilent). The oven temperature was 35 °C. Hydrogen (at a flow rate of 250 ml/min) was used as the carrier gas. Standard curves for the samples from trial 1 were produced using 10%, 30% and 40% methane (Scientific and Technical Gases Ltd.). Standard curves for the samples from trial 2 were produced using 10% and 40% methane (Scientific and Technical Gases Ltd.).

Mean percentage methane could be calculated using equation 1. Each data point could be fitted a particular standard curve, depending on the methane content. The volume of methane produced per gram lyophilised mass (VM, L/g) of feedstock (macroalgae or combined macroalgae and sewage pellets) added could be calculated using equation 2. Biogas volume was the main focus, and data from GC were not analysed in the time available. However, some general observations were noted.

$$PM = \frac{y - c}{m}$$

Equation 1 where:

PM = percentage methane

y = response

c = offset

m = slope of line

$$VM = \left(\frac{PM}{100} \times VG\right) \times 1000$$

Equation 2 where:

VM = volume of methane produced (L/g)

VG = volume of gas produced (L/g)

PM = percentage methane

2.3 Statistical analysis

The null hypotheses are outlined in section 1.7. Data was organised using Microsoft Excel. Statistical analysis, including descriptive statistics, analysis of variance (ANOVA) and Tukey analysis, was done using Minitab® statistical software (version 17) for results as described in chapter 3. Unless otherwise stated, significance is judged at 95 % probability of being different from zero (significance level $\alpha = 0.05$). Effect sizes were not calculated and are not discussed.

For salinity at the start and end of each trial, one-way ANOVA was done with Tukey pairwise comparisons and the P-values were used to determine whether or not the change was significant. (This analysis not done for salinity in trial 1, which showed no change).

As for salinity, it was determined whether or not solids (SS or VSS) changed significantly from the start to the end of each trial. For each trial, ANOVA (general linear model) was also done, with treatment type, bottle number and sample number as factors and start-SS, start-VSS, end-SS and end-VSS as responses. Tukey analysis was then done and P (the adjusted P-value) was used to determine whether or not each factor had a significant effect on each response.

For each trial, ANOVA and Tukey analysis were done as above, in order to test the effect of treatment (digester) type on total biogas volume (day 22). The effect of digester bottle number on total biogas volume was tested in the same way. Using one-way ANOVA with Tukey pairwise comparisons, it was determined whether or not the maximum mean rates of biogas production from different digester types (within each trial) differed significantly from each other. The effect of treatment type and of bottle number on gas per day were tested using ANOVA (general linear model) and Tukey analysis as above.

3 RESULTS

3.1 Feedstocks

The experimental matrix describing digester treatments is shown in table 2.3 in section 2.2.3. Macroalgae (*Laminaria digitata*) is denoted as LD, creamery waste as C and sewage sludge pellets as S, with + indicating a co-digestion.

3.1.1 Macroalgae

Lyophilised mass

The mean lyophilised mass of the macroalgae was 13.01 ± 1.59% of wet mass. Assuming a lyophilised mass of 13.01%, the moisture content was therefore 86.99%.

Solids

Volatile total solids (VTS) in the second subsample of macroalgae had a negative value. There were some issues with the methodology (section 2.1.1), with possible loss of material, but the negative result suggests an issue with recording the mass of the dry crucible, or of the ashed crucible and sample. This subsample was eliminated from the calculation of mean and standard error (SE) for VTS. Because VTS was reported as % of total solids (TS), this subsample was also eliminated in the mean and SE for TS. Mean TS was 920,584 ± 114 mg/kg. Mean VTS was 430,650 ± 218,566 mg/kg (46.8 ± 23.7 % in SS). Given the high mean SE, secondary results could be used.

CHN

The lyophilised, ground macroalgae had a mean %C of 30.04 ± 0.06%. Mean %H was 4.58 ± 0.01%. Mean %N was 1.64 ± 0.02%.

3.1.2 Sewage pellets

Lyophilised mass

The mean lyophilised mass of the sewage pellets was 97.41 ± 0.00% of the mass as provided. (The pellets had already been dried at > 400 °C at the STW but were not subsequently stored in moisture-free conditions). Assuming a lyophilised mass of 97.41%, the moisture content was therefore 2.59%. However, the pellets were particularly hygroscopic and masses were difficult to record after freeze-drying.

Solids

VTS for the first subsample of sewage pellets had a negative value. This suggests similar issues as for macroalgae (section 3.1.1). VTS in the second subsample of pellets was 371.3% in TS, so this was also eliminated. As VTS of the remaining subsample could not be assumed to be representative, all three values were disregarded. Having gone through advanced oxidation, the pellets would be expected to have low VTS content. TS was calculated from all three samples. Mean TS was 958,804 ± 649 mg/L.

CHN

The lyophilised, ground sewage pellets had a mean %C of 40.28 ± 0.06%. Mean %H was 5.76 ± 0.01%. Mean %N was 6.71 ± 0.01%.

Additional analyses (secondary results)

The results of a 2005 laboratory report on pellets from Meary Veg are shown in table 3.1. They are assumed to represent the pellets in the current study. For comparison with the pH determined for creamery waste and digester samples in this study, the pH of the pellets is shown as 6.2.

3.1.3 Creamery waste

Solids

Mean SS content in the creamery waste was 476.0 ± 15.0 mg/L. Mean VSS content was 450.0 ± 15.2 mg/L (94.58 ± 1.71% in SS).

pH

The creamery waste had a pH of 9 ± 0.

Additional analyses (secondary results)

Current average figures relating to waste discharged from the creamery are shown in table 3.2.

Determination	**Result**	**Units**	**Basis**
Total Aluminium	11500	mg/kg	100% DM
Total Iron	7190.0	mg/kg	100% DM
pH	6.2		
Oven Dry Matter	171	g/kg	100% DM
Total Nitrogen (Kjeldahl)	70.5	g/kg	100% DM
Total Phosphorous	19500	mg/kg	100% DM
Total Potassium	6900	mg/kg	100% DM
Total Sulphur	6580	mg/kg	100% DM
Total Magnesium	4320	mg/kg	100% DM
Total Lead	60.9	mg/kg	100% DM
Total Nickel	34.1	mg/kg	100% DM
Total Zinc	218	mg/kg	100% DM
Total Cadmium	2.66	mg/kg	100% DM
Total Chromium	29.5	mg/kg	100% DM
Total Copper	146	mg/kg	100% DM
Total Mercury	1.03	mg/kg	100% DM
Ammonium-N	7030	mg/kg	100% DM

Table 3.1 Results of a laboratory report (from Direct Laboratory Services Ltd in April 2005) on sewage sludge pellets produced at Meary Veg sewage treatment plant. The composition of pellets used in the current study is expected to differ little from the above

Determination	Result	Units
Fat	358	mg/L
Lactic acid	113	mg/L
Solids	9000	mg/L
pH	9.1	

Table 3.2 Current average figures (provided from measurements by workers at the Isle of Man Creamery) relating to creamery waste discharged

3.2 Anaerobic digestion trial 1 (controls and *Laminaria digitata*)

3.2.1 pH

Table 3.3 shows the results for mean pH in each of the digesters at the start and end of trial 1. No change was seen.

Digester	Mean start-pH with mean standard error (SE)	Mean end-pH with mean standard error (SE)
Control 1	8 ± 0	8 ± 0
Control 2	8 ± 0	8 ± 0
Control 3	8 ± 0	8 ± 0
LD1	8 ± 0	8 ± 0
LD2	8 ± 0	8 ± 0
LD3	8 ± 0	8 ± 0

Table 3.3 Results for mean pH in each of the control digesters and the digesters with *Laminaria digitata* (LD) at the start and end of trial 1

3.2.2 Salinity

Table 3.4 shows the results for mean salinity in each of the digesters at the start and end of trial 1. No change was seen. The full results for salinity are in appendix 1.

Digester	**Mean start-salinity (‰) with mean standard error (SE)**	**Mean end-salinity (‰) with mean standard error (SE)**
Control 1	15.00 ± 0.00	15.00 ± 0.00
Control 2	15.00 ± 0.00	15.00 ± 0.00
Control 3	15.00 ± 0.00	15.00 ± 0.00
LD1	15.00 ± 0.00	15.00 ± 0.00
LD2	15.00 ± 0.00	15.00 ± 0.00
LD3	15.00 ± 0.00	15.00 ± 0.00

Table 3.4 Results for mean salinity in each of the control digesters and the digesters with *Laminaria digitata* (LD), at the start and end of trial 1

3.2.3 Solids

Figure 3.1 shows the mean SS in each of the control and *Laminaria digitata* (LD) digesters at the start and end of trial 1. Figure 3.2 shows the mean VSS at the start and end of trial 1, in the same digesters. The full results of solids analyses are shown in appendix 2.

SS (mg/L) in all controls decreased by the end of trial 1, although there is overlap between start-SS and end-SS when SE is considered. Table 3.5 shows the results of one-way ANOVA with Tukey pairwise comparisons, for SS and VSS. Mean VSS decreased from the start to the end of the trial in all controls, with no overlap when SE is considered.

As was seen in the controls, SS in all LD digesters decreased by the end of the trial. For LD2, there is some overlap when SE is considered. LD1 and LD3 showed a larger decrease than did any of the controls. VSS in the LD digesters also decreased from start to end, although there is some overlap in LD2 when SE is considered. As was seen for SS, LD1 and LD3 had a larger decrease in VSS than did the controls.

Digester type	Change in mean SS	Significance of change in mean SS	Change in mean VSS	Significance of change in mean VSS
Controls	Decrease	Significant (P = 0.027)	Decrease	Significant (P = 0.001)
LD	Decrease	Significant (P = 0.008)	Decrease	Significant (P = 0.001)

Table 3.5 Results of one-way analysis of variance (ANOVA) with Tukey pairwise comparisons for mean suspended solids (SS) and mean volatile suspended solids (VSS) in trial 1. The change from start to end is shown for the control digesters and the digesters with *Laminaria digitata* (LD). The significance of each change is indicated by the P-value

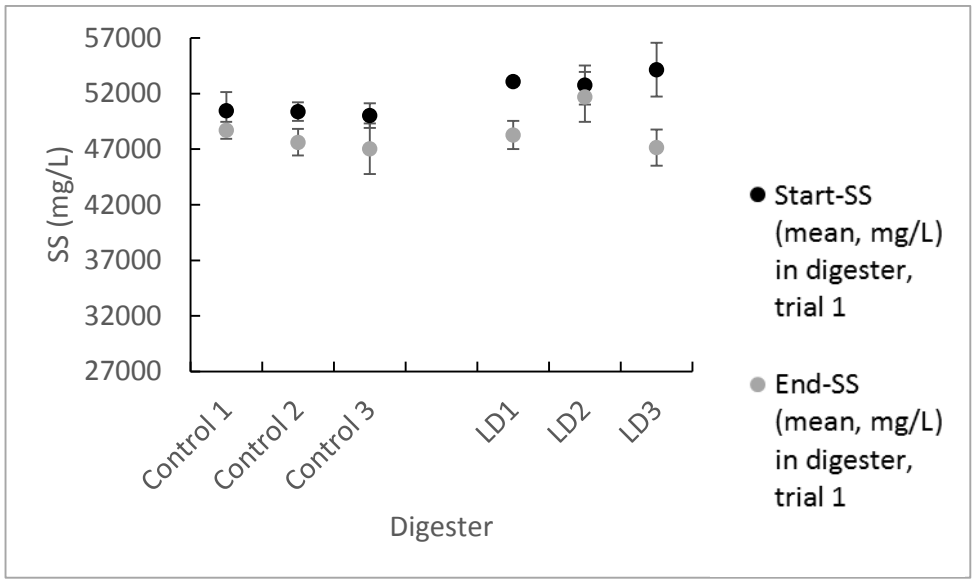

Figure 3.1 Graph showing the mean suspended solids (SS, with mean standard error) in each of the controls and the digesters with *Laminaria digitata* (LD) at the start and end of trial 1. (SS decreased in all digesters, although some overlap is seen in control 1, control 3 and LD2 when SE is considered. LD1 and LD3 showed a greater decrease than did the controls)

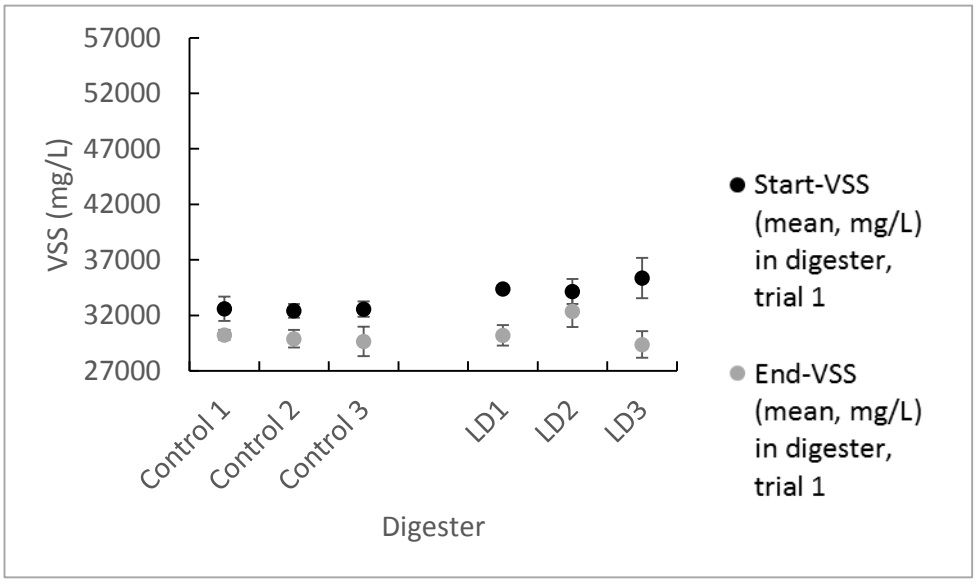

Figure 3.2 Graph showing the mean volatile suspended solids (VSS, with mean standard error) in each of the controls and the digesters with *Laminaria digitata* (LD) at the start and end of trial 1. (VSS decreased in all digesters, although some overlap is seen in LD2 when SE is considered. LD1 and LD3 showed a greater decrease than did the controls)

Table 3.6 shows the results of ANOVA and Tukey analysis relating to the effect of various factors on SS and VSS in trial 1. The LD digesters had 1 g/L lyophilised macroalgae added, so would be expected to have more initial solids than the controls. Macroalgae produced a significant increase in start-SS and in start-VSS, but treatment type had no significant effect on end-SS or on end-VSS.

		FACTOR		
		Treatment type (Control or LD)	**Bottle number (1, 2 or 3)**	**Sample number (1, 2 or 3)**
RESPONSE	**Start-SS**	Significant (P = 0.024)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
	End-SS	Not significant (P = 0.410)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
	Start-VSS	Significant (P = 0.024)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
	End-VSS	Not significant (P = 0.458)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)

Table 3.6 Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for solids in trial 1. Factors tested were treatment type, bottle number and sample number. (Treatment types were controls and 1 g/L *Laminaria digitata* (LD)). Responses tested were suspended solids (SS) and volatile suspended solids (VSS) at the start and end. The significance of each effect is indicated by the adjusted P-value

3.2.4 Temperature of the water bath

The mean daily temperature of the water bath during trial 1 was 34.95 ± 0.03 °C.

3.2.5 Mean sea-level pressure

The MSLP (Met Office) values noted at the time of cylinder readings during trial 1 had a mean of 1013.8 ± 1.36 hPa. The minimum was 1001 hPa (day 15). The maximum was 1026 hPa (day 10). Readings from the control cylinder (without a digester) in trial 1 were plotted against MSLP. There was a slight negative correlation. R^2 for the trend line (linear or polynomial) was 0.5. The full results for MSLP are in appendix 3.

3.2.6 Volume and rate of biogas production

Figure 3.3 shows the mean cumulative biogas production from each anaerobic digester type in trial 1. The full results for cumulative biogas production are shown in appendix 4.

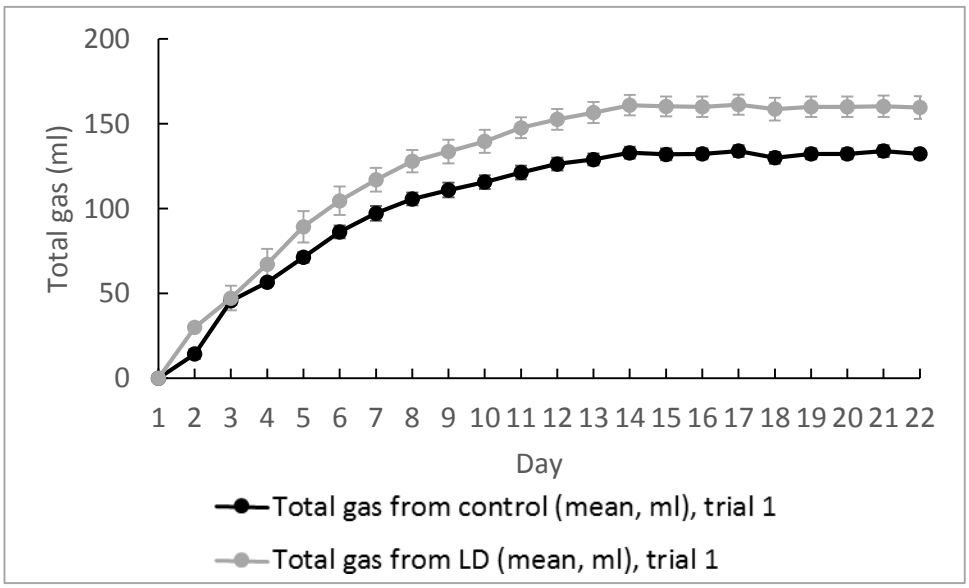

Figure 3.3 Graph showing the mean cumulative biogas production (with mean standard error) from the controls and the digesters with *Laminaria digitata* (LD) in trial 1. (The digesters with LD produced significantly more gas in total than did the controls. However, the controls produced a reasonable volume despite no feeding)

There were issues with agitation and some loss of gas from days 1 to 3, followed by a change in methodology (see section 2.2.5). However, this was thought to be a minor issue. There was possibly some gas loss also from leaving the caps off digesters whilst aliquots were extracted. For subsequent trials, this process was quicker. Cylinder readings were taken before and after agitating the digesters from day 1 to day 3, but only pre-agitation readings were used. Agitation was stopped from day 4 onwards.

The mean total volumes of gas on day 22 in trial 1 are summarised in table 3.7. The controls produced a reasonable amount of gas despite no feeding with macroalgae. The difference between the mean LD total and the mean control total was only 2 ml more than the difference between the highest- and lowest-producing LD digesters.

Mean total gas volume (ml) with mean standard error (SE)	
Controls (1, 2 and 3)	**LD (1, 2 and 3)**
132.33 ± 3.18	159.67 ± 6.69

Table 3.7 Mean total volume of biogas (day 22) produced by the control digesters and the digesters with *Laminaria digitata* (LD), in trial 1

Table 3.8 shows the results of ANOVA and Tukey analysis relating to the effect of treatment type and bottle number on total biogas volume in trial 1. The full results of these analyses on total gas are in appendix 6. The addition of macroalgae increased total volume.

Effect of treatment type on total volume of biogas	**Effect of bottle number on total volume of biogas**
Significant (P = 0.021)	Not significant (P > 0.05)

Table 3.8 Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for total biogas in trial 1. Factors tested were treatment type and bottle number. (Treatment types were controls and 1 g/L *Laminaria digitata* (LD)). The response was total volume of gas. The significance of the effect is indicated by the adjusted P-value

Figure 3.4 shows the mean volume of biogas produced per day by each digester type in trial 1. The rate of gas production increased on day 5 and tended to decrease or remain similar from day 5 onwards. Agitation produced a rapid initial increase in gas production. Some negative readings were noted. By the second week of the trial, gas production was slowing down. It was thought that this might be a lag phase, the soluble sugars in the macroalgae being digested first, followed by alginate (e.g. Horn and Østgaard, 2001; Østgaard *et al.*, 1993). However, no second phase of gas production was apparent during the 21 days, and further gas samples were not taken. Table 3.9 shows the mean maximum daily volume of gas produced by each of the groups of digesters in trial 1, and the day on which each maximum was reached. The highest rate of production for each of the controls was seen between day 2 and day 3. The LD digesters reached peak rate before the controls, between day 1 and day 2. One-way ANOVA with Tukey pairwise comparison showed no significant difference between these two maximum mean rates (P = 0.435).

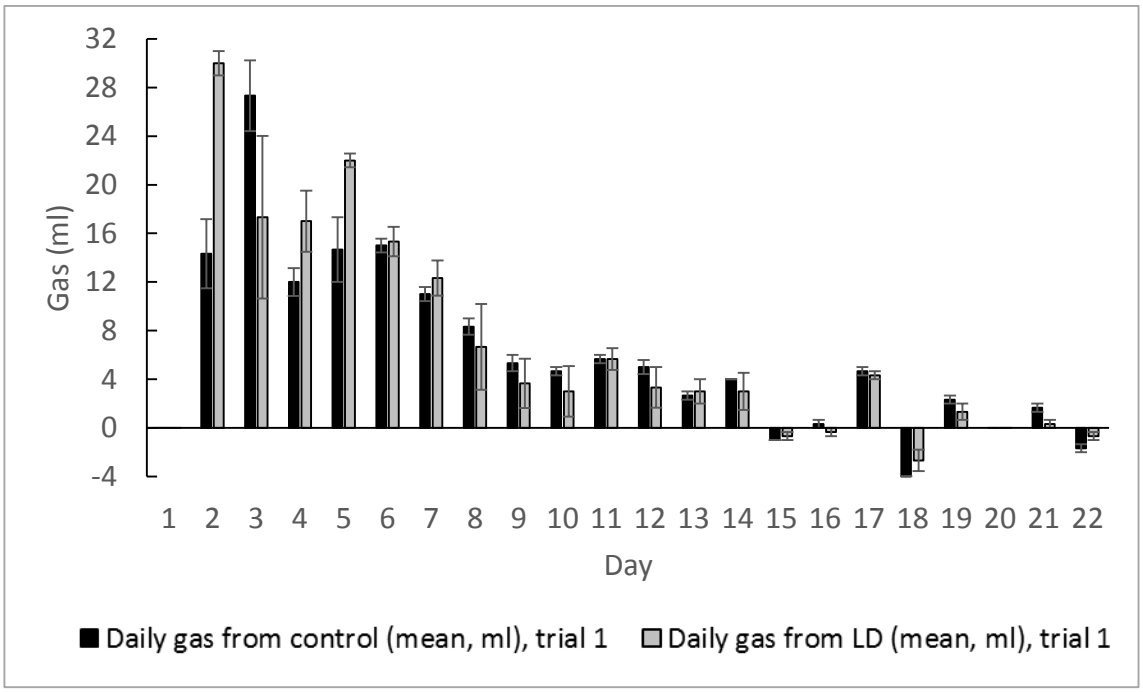

Figure 3.4 Bar chart showing the mean biogas production per day (with mean standard error) from the controls and the digesters with *Laminaria digitata* (LD) in trial 1. (After an initial increase in gas production, the rate tended to decrease or stay similar from day 5 onwards. The controls reached mean maximum on day 3. The LD digesters reached mean maximum on day 2)

Mean maximum daily gas volume (ml) with mean standard error (SE)	
Controls (1, 2 and 3)	**LD (1, 2 and 3)**
27.33 ± 2.91 (Day 2-3)	30.00 ± 1.00 (Day 1-2)

Table 3.9 Results for mean maximum daily biogas volume produced by the control digesters and the digesters with *Laminaria digitata* (LD), in trial 1. The days corresponding to each maximum are shown

Table 3.10 shows the results of ANOVA and Tukey analysis relating to the effect of treatment type and bottle number on daily biogas volume in trial 1. The full results of these analyses on daily gas are in appendix 7.

Effect of treatment type on daily volume of biogas	**Effect of bottle number on daily volume of biogas**
Not significant (P = 0.711)	Not significant (P > 0.05)

Table 3.10 Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for daily biogas in trial 1. Factors tested were treatment type and bottle number. (Treatment types were controls and 1 g/L *Laminaria digitata* (LD)). The response was total volume of gas. The significance of the effect is indicated by the adjusted P-value

It was noted that more water had evaporated from the tub with the control cylinder and beehive shelf (with no digester, section 2.2.10) than from the others. The reason for this is not clear, although this tub was next to the heated water bath. Tubs with control digesters were nearer to the window than those with LD digesters, and more water evaporated from the former. However, evaporation of water from tubs was not expected to affect cylinder readings.

3.2.7 Methane content of biogas

Methane was detected in the gas samples from both the controls and LD digesters in trial 1. However, there were issues with digesters being uncapped at intervals when the digesters were agitated on day 1 to day 3 (section 2.2.5). Sample replication was reasonably good. However, the gas on some sampling days may have been affected by tubing being left temporarily open to the air, particularly in the earliest phase of the trial, before gas in tubing was pushed out by subsequent production. The standards used for calibration were 10% and 30% methane. All methane contents determined were lower than these levels and therefore lower than expected.

3.3 Anaerobic digestion trial 2 (controls; creamery waste co-digested with *Laminaria digitata*; and sewage pellets co-digested with *Laminaria digitata*)

3.3.1 pH

Table 3.11 shows the results for mean pH in each of the digesters at the start and end of trial 2. Identical results were obtained.

Digester	**Mean start-pH with mean standard error (SE)**	**Mean end-pH with mean standard error (SE)**
Control 1	8 ± 0	8 ± 0
Control 2	8 ± 0	8 ± 0
Control 3	8 ± 0	8 ± 0
C+LD1	8 ± 0	8 ± 0
C+LD2	8 ± 0	8 ± 0
C+LD3	8 ± 0	8 ± 0
S+LD1	8 ± 0	8 ± 0
S+LD2	8 ± 0	8 ± 0
S+LD3	8 ± 0	8 ± 0

Table 3.11 Results for mean pH in each of the control digesters; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD), at the start and end of trial 2

3.3.2 Salinity

Figure 3.5 shows the mean start-salinity and end-salinity in each of the digesters in trial 2. The full results of salinity analyses are shown in appendix 1.

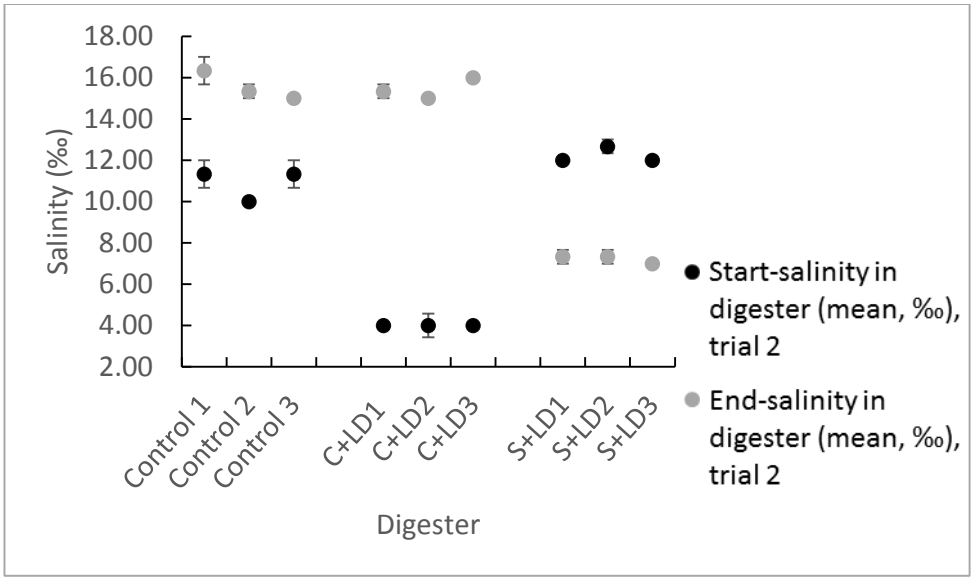

Figure 3.5 Graph showing the mean salinity (with mean standard error) in each of the controls; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD), at the start and end of trial 2. (Salinity increased in the controls and C+LD digesters but decreased in the S+LD digesters. C+LD showed the greatest change)

Salinity in all controls and all C+LD digesters increased by the end of the trial. C+LD produced the greatest increase in salinity. In contrast to the controls and the C+LD digesters, the S+LD digesters had a decrease in salinity by the end of the trial. End-salinity in S+LD still exceeded start-salinity in C+LD. The decrease in salinity in S+LD was smaller than the increase in C+LD. Table 3.12 shows the results of one-way ANOVA with Tukey pairwise comparisons, for salinity from the start to the end of trial 2. Start-salinity was significantly higher in the controls than in C+LD ($P = 0.000$). Start-salinity in S+LD was significantly higher than in the controls ($P = 0.003$). There was no significant difference between end-salinity in the controls and C+LD ($P = 0.750$). However, end-salinity in the controls was significantly higher than that in S+LD ($P = 0.000$). End-salinity in C+LD was significantly higher than that in S+LD ($P = 0.000$).

Digester type	Change in mean salinity	Significance of change in mean salinity
Controls	Increase	Significant (P = 0.000)
C+LD	Increase	Significant (P = 0.000)
S+LD	Decrease	Significant (P = 0.000)

Table 3.12 Results of one-way analysis of variance (ANOVA) with Tukey pairwise comparisons for salinity in trial 2, within each digester type. The change from start to end is shown for the control digesters; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD). The significance of each change is indicated by the P-value

3.3.3 Solids

Figure 3.6 shows the mean start-SS and end-SS in each of the digesters in trial 2. Figure 3.7 shows the mean start-VSS and end-VSS in trial 2, in the same digesters. The full results of solids analyses are shown in appendix 2.

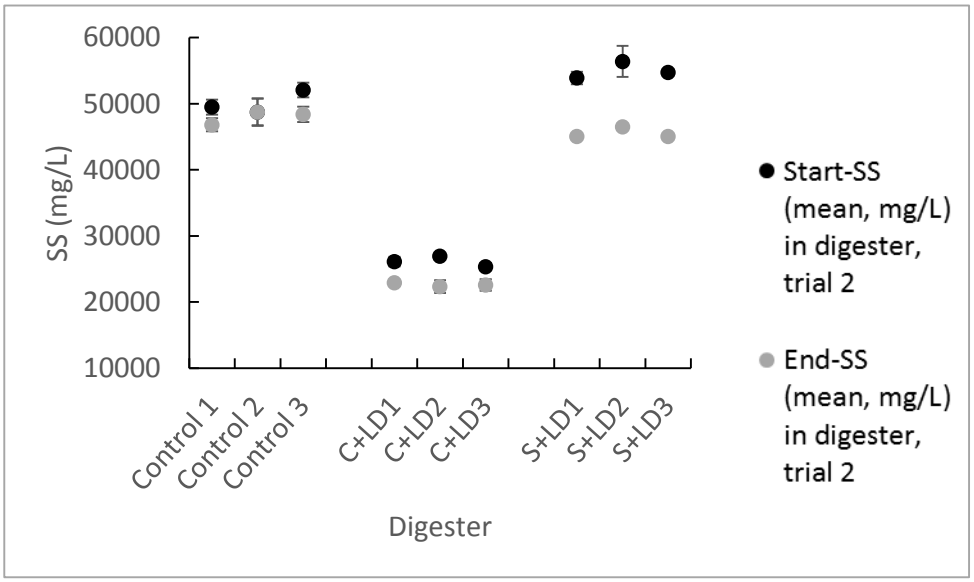

Figure 3.6 Graph showing the mean suspended solids (SS, with mean standard error) in each of the controls; the digesters with creamery waste co-digested with *Laminaria digitata* (C+LD); and the digesters with sewage pellets co-digested with *Laminaria digitata* (S+LD), at the start and end of trial 2. (SS decreased in all digesters, but the decrease in control 2 was less than SE. The clearest decrease was in the S+LD digesters. C+LD had the lowest SS at the start and end)

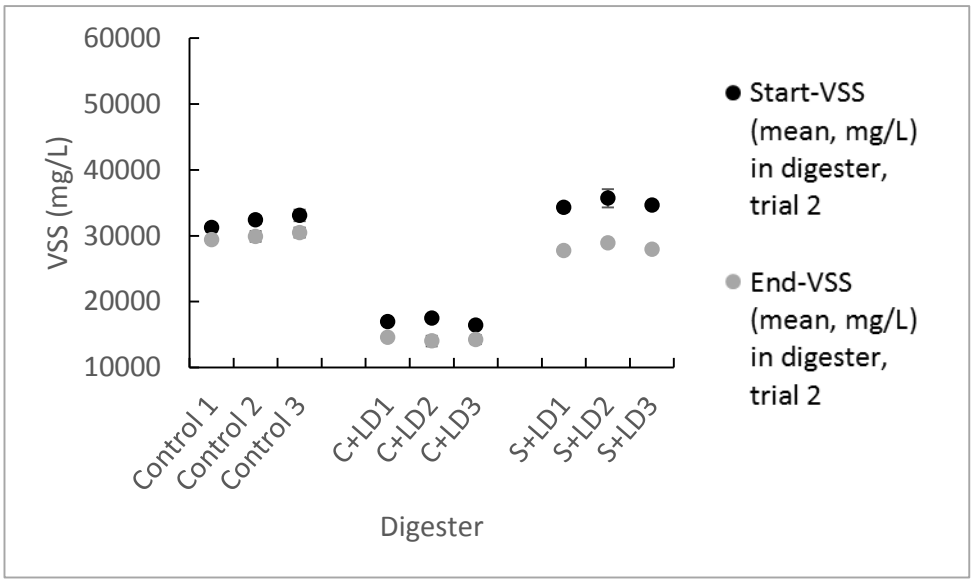

Figure 3.7 Graph showing the mean volatile suspended solids (VSS, with mean standard error) in each of the controls; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD), at the start and end of trial 2. (VSS decreased in all digesters, but with some overlap in control 1 when SE is considered. The S+LD digesters showed the clearest decrease)

SS in all digesters in trial 2 decreased by the end of the trial, even when SE is considered, except in control 2, in which the decrease was less than SE. The clearest decrease was in the S+LD digesters, followed by the C+LD digesters. C+LD had lower initial SS and VSS content than the other two groups at both the start and end. VSS is all digesters decreased from start to end. The clearest decrease was in S+LD. There was some overlap between start-VSS and end-VSS in control 1 when SE is considered.

Table 3.13 shows the results of one-way ANOVA with Tukey pairwise comparisons, for SS and VSS.

Digester type	**Change in mean SS**	**Significance of change in mean SS**	**Change in mean VSS**	**Significance of change in mean VSS**
Controls	Decrease	Not significant (P = 0.071)	Decrease	Significant (P = 0.045)
C+LD	Decrease	Significant (P = 0.000)	Decrease	Significant (P = 0.000)
S+LD	Decrease	Significant (P = 0.000)	Decrease	Significant (P = 0.000)

Table 3.13 Results of one-way analysis of variance (ANOVA) with Tukey pairwise comparisons for mean suspended solids (SS) and mean volatile suspended solids (VSS) in trial 2. The changes from start to end are shown for the control digesters; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD). The significance of each change is indicated by the P-value

Table 3.14 shows the results of ANOVA and Tukey analysis relating to the effect of various factors on SS and VSS in trial 2. S+LD had the highest mean start-SS, followed by the controls and then C+LD. The controls had the highest mean end-SS, followed by S+LD and then C+LD. S+LD had the highest mean start-VSS, followed by the controls and then C+LD. The controls had the highest mean end-VSS, followed by S+LD and then C+LD.

		FACTOR		
		Treatment type	**Bottle number**	**Sample number**
RESPONSE	**Start-SS**	Significant (P = 0.000 for all differences of means)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
	End-SS	Significant (P < 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
	Start-VSS	Significant (P = 0.000 for all differences of means)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
	End-VSS	Significant (P < 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)

Table 3.14 Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for solids in trial 2. Factors tested were treatment type, bottle number and sample number. (Treatment types were controls; co-digestion of creamery waste and *Laminaria digitata* (C+LD); and co-digestion of sewage pellets and *Laminaria digitata* (S+LD). Responses tested were suspended solids (SS) and volatile suspended solids (VSS) at the start and end. The significance of each effect is indicated by the adjusted P-value

3.3.4 Temperature of the water bath

The mean daily temperature of the water bath during trial 2 was 34.74 ± 0.08 °C.

3.3.5 Mean sea-level pressure

The MSLP (Met Office) values noted at the time of cylinder readings during trial 2 had a mean of 1010.9 ± 1.42 hPa. The minimum was 998.0 hPa (day 5). The maximum was 1023.0 (day 21 and day 22). The full results for MSLP are in appendix 3.

3.3.6 Volume and rate of biogas production

Figure 3.8 shows the mean cumulative biogas production from each anaerobic digester type in trial 2. The full results for cumulative biogas production are shown in appendix 4.

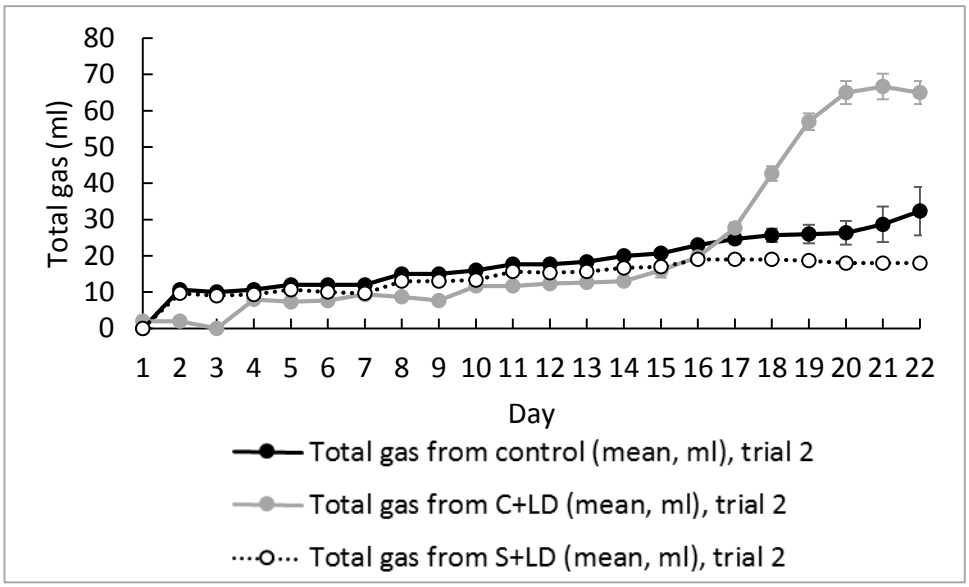

Figure 3.8 Graph showing the mean cumulative biogas production (with mean standard error) from the controls; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD) in trial 2. (There were problems with backflow and gas did not immediately show in the cylinders. C+LD showed a sharp increase in production around day 17 and produced most overall)

Adjustments to agitation in relation to backflow problems in trial 2 are outlined in section 2.2.5. Daily agitation after the start helped increasingly. Flow began later to generally move more in the right direction. However, production of biogas did not immediately show in the cylinders, even when liquid was being pushed out of tubing. Even given agitation of the digesters, permanganate and water that had been sucked towards them took time to be pushed out again. When agitation had been continued, most digesters had a small amount of inoculum

pushed down the tubing away from them. Some inoculum had reached the stopcocks. The process eventually improved in terms of data output, with bubbles appearing in the cylinders. By the end of the trial, there was still some residual liquid in the tubing. However, the replicates of each digester type (other than one of the controls towards the end) behaved in a consistent way, so it was thought that any differences between amounts of liquid were not problematic for the data. The digesters were still forming bubbles by the end of the trial, although this was not necessarily showing in the data. Due to condensation or inoculum in tubing, some gas samples were reduced in volume or omitted, but the biogas production data in trial 2 was corrected accordingly. Gas was sampled on days 3, 7, 10 and 13 in this trial. There was still some backflow immediately after sampling gas. It appears that some of the gas production suggested by the data would not have shown up had readings not been corrected for gas sampled.

The mean total volumes of gas on day 22 in trial 2 are summarised in table 3.15. Although the total volume from the controls was between that from C+LD and S+LD, it was noted that one or two of the controls seemed to increase their production near the end of the trial. One of the S+LD digesters in particular appeared to be relatively inactive. A blockage of inoculum in the tubing outlet from the digester may have been an issue.

Mean total gas volume (ml) with mean standard error (SE)		
Controls (1, 2 and 3)	**C+LD (1, 2 and 3)**	**S+LD (1, 2 and 3)**
32.33 ± 6.64	65.00 ± 3.21	18.00 ± 0.00

Table 3.15 Mean total volume of biogas (day 22) produced by the control digesters; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD), in trial 2

Table 3.16 shows the results of ANOVA and Tukey analysis relating to the effect of treatment type and bottle number on total biogas volume in trial 2. The full results of these analyses on total gas are in appendix 6.

Effect of treatment type on total volume of biogas	**Effect of bottle number on total volume of biogas**
Significant for C+LD compared to controls (P = 0.004)	Not significant (P > 0.05 for all differences of means)
Significant for C+LD compared to S+LD (P = 0.001)	
Not significant for S+LD compared to controls (P = 0.119)	

Table 3.16 Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for total biogas in trial 2. Factors tested were treatment type and bottle number. (Treatment types were controls; co-digestion of creamery waste and *Laminaria digitata* (C+LD); and co-digestion of sewage pellets and *Laminaria digitata* (S+LD). The response was total volume of gas. The significance of the effect is indicated by the adjusted P-value

Figure 3.9 shows the mean biogas produced per day by each digester type in trial 2. Table 3.17 shows the mean maximum daily volume of gas produced by each of the groups of digesters in trial 2, and the day on which each maximum was reached. The highest rate of production for each control was between day 1 and day 2. After an initial lag, the rate of production from the C+LD digesters increased sharply around day 16. Gas was easily released from the C+LD digesters by agitation. C+LD1 and C+LD3 both had their highest rate of production between day 15 and 16. The maximum rate for C+LD2 was between day 15 and 16 as well as between day 16 and day 17. Like the controls, S+LD1, S+LD2 and S+LD3 had their highest rate of biogas production between day 1 and day 2. One-way ANOVA with Tukey pairwise comparison showed a significant difference between the maximum mean rates for the controls and C+LD (P = 0.008). There was no significant difference between the controls and S+LD (P = 0.251).

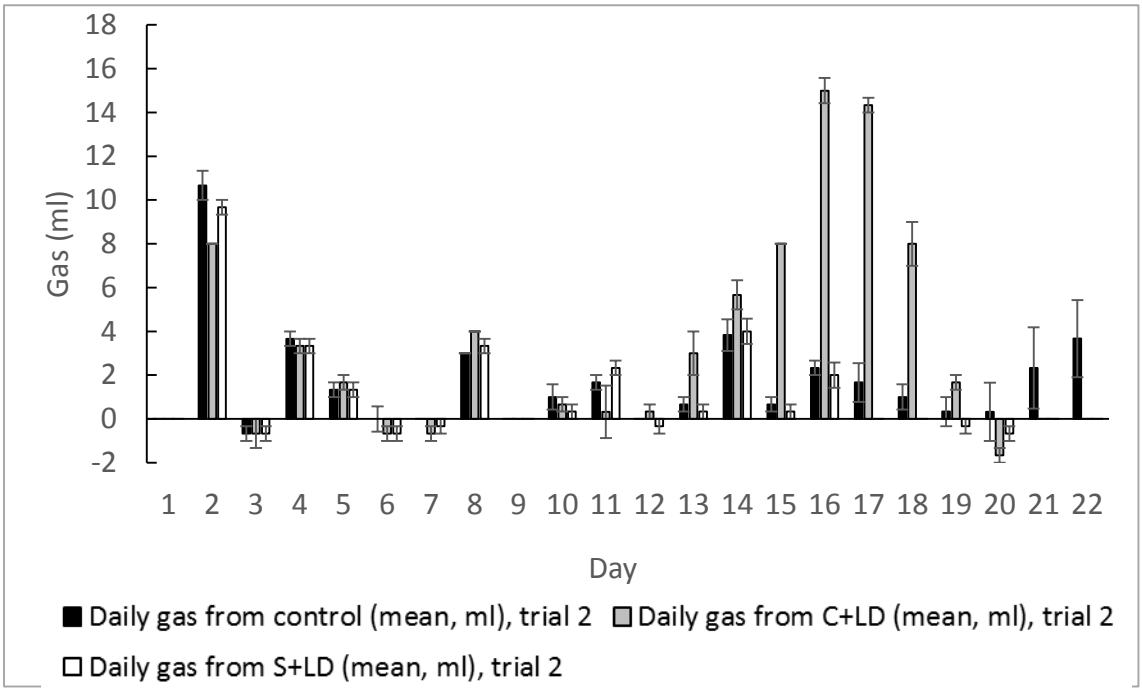

Figure 3.9 Bar chart showing the mean biogas production per day (with mean standard error) from the controls; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD), in trial 2. (The controls and S+LD digesters peaked on day 2. After an initial lag, the rate of production from the C+LD digesters increased sharply around day 16)

Mean maximum daily gas volume (ml) with mean standard error (SE)		
Controls (1, 2 and 3)	**C+LD (1, 2 and 3)**	**S+LD (1, 2 and 3)**
10.67 ± 0.67 (Day 1-2)	15.00 ± 0.58 (Day 15-16)	9.67 ± 0.33 (Day 1-2)

Table 3.17 Results for mean maximum daily biogas volume produced by the control digesters; the digesters with creamery waste and *Laminaria digitata* (C+LD); and the digesters with sewage pellets and *Laminaria digitata* (S+LD), in trial 2. The days corresponding to each maximum are shown

Table 3.18 shows the results of ANOVA and Tukey analysis relating to the effect of treatment type and bottle number on daily biogas volume in trial 2. The full results of these analyses on daily gas are in appendix 7.

Effect of treatment type on daily volume of biogas	**Effect of bottle number on daily volume of biogas**
Significant for controls compared to C+LD (P = 0.004)	Not significant (P > 0.05)
Significant for C+LD compared to S+LD (P = 0.001)	
Not significant for S+LD compared to controls (P = 0.119)	

Table 3.18 Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for daily biogas in trial 2. Factors tested were treatment type and bottle number. (Treatment types were controls; co-digestion of creamery waste and *Laminaria digitata* (C+LD); and co-digestion of sewage pellets and *Laminaria digitata* (S+LD)). The response was total volume of gas. The significance of the effect is indicated by the adjusted P-value

3.3.7 Methane content of biogas

Methane was detected in the gas samples from trial 2. Sample replication was good, but differences between digester types were only very subtle. The standards used for calibration were 10% and 40% methane. All methane contents determined were lower than these levels and therefore lower than expected. Methane content was also lower in general than in trial 1, with many of the trial 2 samples below the 10% standard. In trial 2, the digesters were not agitated the same way as in trial 1, so there were no issues with air in tubing.

3.4 Anaerobic digestion trial 3 (controls; creamery waste co-digested with 1 g/L *Laminaria digitata*; and creamery waste co-digested with 2 g/L *Laminaria digitata*)

3.4.1 pH

Table 3.19 shows the results for mean pH in each of the digesters at the start and end of trial 3. Identical results were obtained.

Digester	Mean start-pH with mean standard error (SE)	Mean end-pH with mean standard error (SE)
Control 1	8 ± 0	8 ± 0
Control 2	8 ± 0	8 ± 0
Control 3	8 ± 0	8 ± 0
C+LD.A1	8 ± 0	8 ± 0
C+LD.A2	8 ± 0	8 ± 0
C+LD.A3	8 ± 0	8 ± 0
C+LD.B1	8 ± 0	8 ± 0
C+LD.B2	8 ± 0	8 ± 0
C+LD.B3	8 ± 0	8 ± 0

Table 3.19 Results for mean pH in each of the control digesters; the digesters with creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and the digesters with creamery waste and 2 g/L *Laminaria digitata* (C+LD.B), at the start and end of trial 3

3.4.2 Salinity

Figure 3.10 shows the mean start-salinity and end-salinity in each of the digesters in trial 3. Salinity in all digesters increased between the start and end of the trial. However, the difficulty and precision of refractometer readings and calibration at different times mean that the differences between results over this narrow range of low salinity (0-2‰) may indicate little. The full results for salinity are in appendix 1.

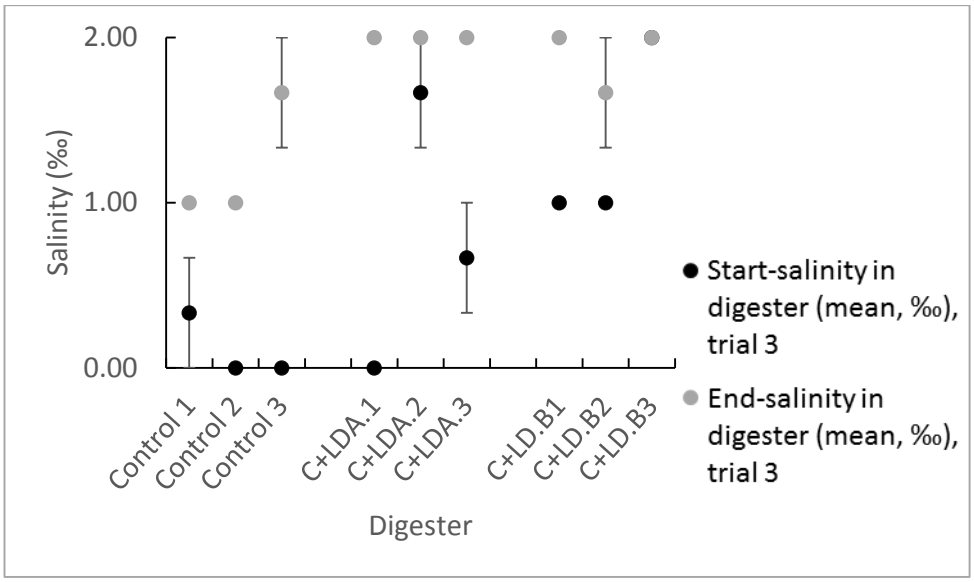

Figure 3.10 Graph showing the mean salinity (with mean standard error) in each of the control digesters; the digesters with creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and the digesters with creamery waste and 2 g/L *Laminaria digitata* (C+LD.B), at the start and end of trial 3. (The results were low and narrow-ranging, and may indicate little)

3.4.3 Solids

Figure 3.11 shows the mean start-SS and end-SS in each of the digesters in trial 3. Figure 3.12 shows the start-VSS and end-VSS in trial 3, in the same digesters. The full results for solids are in appendix 2.

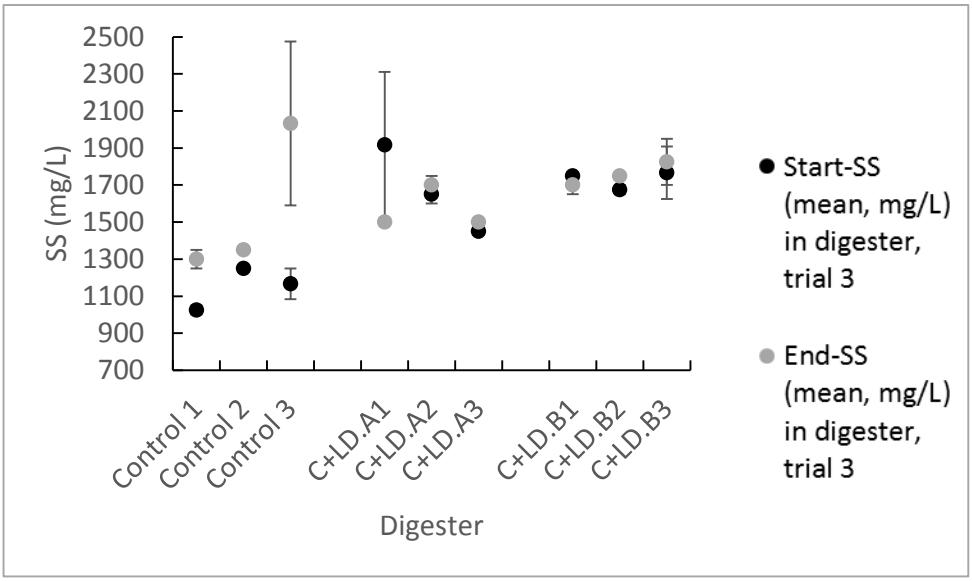

Figure 3.11 Graph showing the mean suspended solids (SS, with mean standard error) in each of the control digesters; the digesters with creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and the digesters with creamery waste and 2 g/L *Laminaria digitata* (C+LD.B), at the start and end of trial 3. (SS in the controls increased. SS in some of the C+LD.A and C+LD.B digesters increased while SS in other digesters decreased, but with some overlap when standard error is considered)

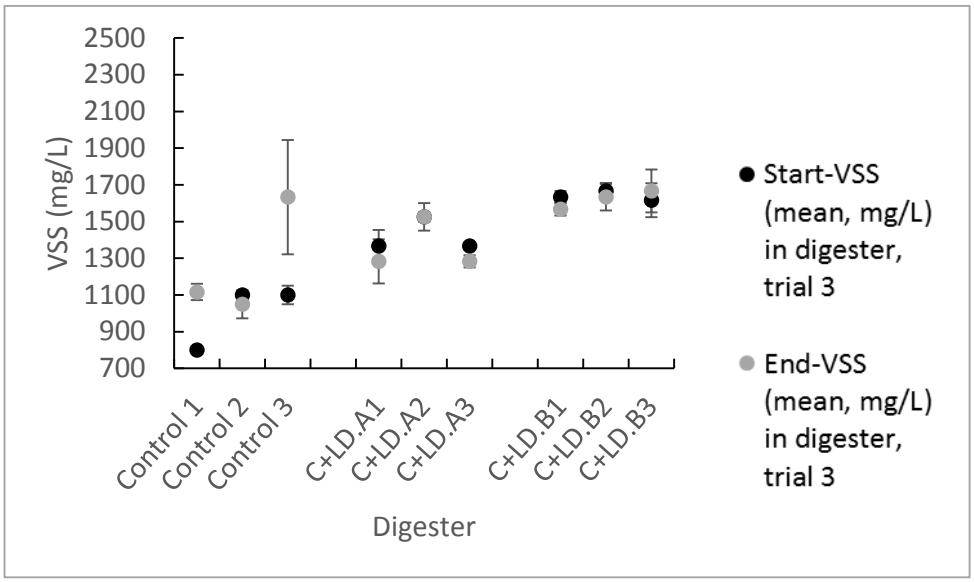

Figure 3.12 Graph showing the mean volatile suspended solids (VSS, with mean standard error) in each of the control digesters; the digesters with creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and the digesters with creamery waste and 2 g/L *Laminaria digitata* (C+LD.B), at the start and end of trial 3. (VSS in two of the controls, and one of the C+LD.B digesters increased. VSS in other digesters decreased or did not change. However, there is some overlap when standard error is considered)

For trial 3, the following samples were removed from the dataset for start solids values, because they gave values for start-VSS in excess of the end-SS values from which they were calculated: control 1, sample 3; control 2, sample 1 and sample 3 (i.e. a single value was used); control 3, sample 3; and C+LD.A2, sample 2. C+LD.A2, sample 1 gave a value for end-VSS in excess of the end-SS value from which it was calculated. This sample was removed from the dataset for end solids values. There may have been issues with transfer of material during analysis of samples, which had higher creamery waste content and contained fewer solids than samples in the other trials.

SS in the controls increased by the end of trial 3. Control 3 had a high mean and SE for end-SS. VSS increased in control 1 and control 3 from start to end, although the mean and SE for end-VSS in control 3 were high. The mean end value for VSS was slightly higher than the start.

SS in C+LD.A1 decreased by the end of the trial, but the SE for start-SS was high. SS in C+LD.A2 and C+LD.A3 increased slightly, although there is some overlap when SE is considered. In C+LD.A, end-SS was slightly lower than start-SS. VSS decreased in C+LD.A1 and C+LD.A3 decreased from the start to the end of trial 3, although there is some overlap in C+LD.A1 when SE is considered. There was no change in C+LD.A2, but only when SE is not considered. In the C+LD.A digesters, the mean end value was slightly lower than the start.

SS in C+LD.B1 decreased from the start to the end of trial 3, whereas SS in C+LD.B2 and C+LD.B3 increased. However, there is overlap for C+LD.B1 and C+LD.B3 when SE is considered. In the C+LD.B digesters, the mean end value was slightly lower than the start. VSS in C+LD.B1 and C+LD.B3 decreased slightly from the start to the end of the trial, whereas VSS in C+LD.B2 increased slightly. However, there is overlap between start-VSS and end-VSS for each digester when SE is considered. In the C+LD.B digesters, the mean end value was slightly lower than the start.

Table 3.20 shows the results of one-way ANOVA with Tukey pairwise comparisons, for SS and VSS.

Digester type	**Change in mean SS**	**Significance of change in mean SS**	**Change in mean VSS**	**Significance of change in mean VSS**
Controls	Increase	Significant (P = 0.022)	Increase	Not significant (P = 0.152)
C+LD.A	Decrease	Not significant (P = 0.619)	Decrease	Not significant (P = 0.391)
C+LD.B	Decrease	Not significant (P = 0.399)	Decrease	Not significant (P = 0.761)

Table 3.20 Results of one-way analysis of variance (ANOVA) with Tukey pairwise comparisons for mean suspended solids (SS) and mean volatile suspended solids (VSS) in trial 3. The changes from start to end are shown for the control digesters; the digesters with creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and the digesters with creamery waste and 2 g/L *Laminaria digitata* (C+LD.B). The significance of each change is indicated by the P-value

Table 3.21 shows the results of ANOVA and Tukey analysis relating to the effect of various factors on SS and VSS in trial 3. For end-SS, C+LD.B had the highest mean value, followed by C+LD.A and then the controls, which would be expected given the masses of solids added (or not added).

For start-VSS, C+LD.B had the highest mean, followed by C+LD.A and then the controls, which would be expected given the masses of solids added (or not added). C+LD.B had the highest mean end-VSS, followed by C+LD.A and then the controls, as expected given the solids added (or not added).

		FACTOR		
		Treatment type	**Bottle number**	**Sample number**
RESPONSE	**Start-SS**	Not significant for C+LD.A vs. C+LD.B (P = 0.656)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
		Significant for controls vs. C+LD.A (P = 0.001)		
		Significant for controls vs. C+LD.B (P = 0.000)		
	End-SS	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
	Start-VSS	Significant (P < 0.05 for all differences of means)	Significant for bottle 2 vs. 1 (P = 0.046)	Significant for the difference between bottle 2 and 1 (P = 0.049)
			Not significant for bottle 3 vs. 1 (P > 0.05)	Not significant for the difference between bottle 3 and 1 (P > 0.05)
			Not significant for bottle 3 vs. 2 (P > 0.05)	Not significant for the difference between bottle 3 and 2 (P > 0.05)
	End-VSS	Not significant for C+LD.A vs. C+LD.B (P = 0.086)	Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05 for all differences of means)
		Not significant for controls vs. C+LD.A (P = 0.864)		
		Significant for controls vs. C+LD.B (P = 0.025)		

Table 3.21 (above) Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for solids in trial 3. Factors tested were treatment type, bottle number and sample number. (Treatment types were controls; co-digestion of creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and co-digestion of creamery waste and 2 g/L *Laminaria digitata* (C+LD.B). Responses tested were suspended solids (SS) and volatile suspended solids (VSS) at the start and end. The significance of each effect is indicated by the adjusted P-value

3.4.4 Temperature of the water bath

The mean daily temperature of the water bath during trial 3 was 34.75 ± 0.08 °C.

3.4.5 Mean sea-level pressure

The MSLP (Met Office) values noted at the time of cylinder readings during trial 3 had a mean value of 1005.70 ± 1.82 hPa. The minimum was 988.0 hPa (day 7). The maximum was 1024.0 hPa (day 1). The full results for MSLP are in appendix 3.

3.4.6 Volume and rate of biogas production

Figure 3.13 shows the mean cumulative biogas production from each anaerobic digester type in trial 3. The full results of cumulative gas production are in appendix 4.

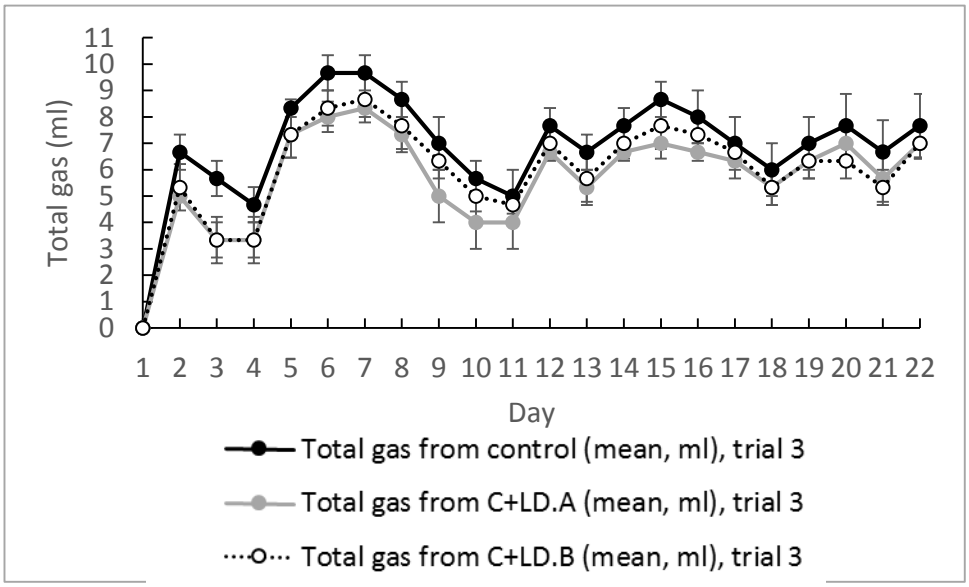

Figure 3.13 Graph showing the mean cumulative biogas production (with mean standard error) from the controls; the digesters with 1 g/L *Laminaria digitata* (C+LD.A) and the digesters with 2 g/L *Laminaria digitata* (C+LD.B) in trial 3. (The results were severely affected by backflow)

Issues with backflow were noted on day 3 of trial 3. The point at which issues began is unclear. After being agitated on day 2, the digesters were next checked on day 3, when it was seen that some potassium permanganate had been sucked out of the scrubbers and into some of the digesters. There had been no immediately obvious problems on day 1 or day 2. Daily notes were made on the movement of permanganate before, during and after agitation. Some of the digesters still produced gas after influx of permanganate, despite this being an antibiotic. However, this gas production showed up little in the data. Agitation sometimes had the opposite effect of worsening backflow, at least temporarily. By around day 18, despite daily agitation of digesters, the data consisted basically of permanganate moving back and forth. There were still no bubbles showing in cylinders, although some digesters still seemed to be producing a small amount of gas. The general pattern was that agitation pushed permanganate away from the digesters and then, after agitation, the permanganate further towards them immediately but more slowly than it had been pushed away. The next day, permanganate tended to have been pushed away from the digesters again. There was later less suction of permanganate completely into the bottles than previously, although this still occurred occasionally. Sometimes the permanganate moved tens of centimetres into the digesters

within minutes, due to movement of bubble wrap and tubing at times of readings and agitation. By the following week, there was no real change from the previous data. Permanganate was still moving back and forth. Gas was still not showing in cylinders, although several bubbles were seen moving through tubing and scrubbers or staying trapped. No gas was sampled in trial 3, due to backflow being more severe than in previous trials.

The mean total volumes of gas on day 22 in trial 3 are summarised in table 3.22.

Mean total gas volume (ml) with mean standard error (SE)		
Controls (1, 2 and 3)	**C+LD.A (1, 2 and 3)**	**C+LD.B (1, 2 and 3)**
7.67 ± 1.20	7.00 ± 0.58	7.00 ± 0.58

Table 3.22 Mean total volume of biogas (day 22) produced by the control digesters; the digesters with creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and the digesters with sewage pellets and 2 g/L *Laminaria digitata* (C+LD.B), in trial 3

Table 3.23 shows the results of ANOVA and Tukey analysis relating to the effect of treatment type and bottle number on total biogas volume in trial 3. The full results of these analyses on total gas are in appendix 6.

Effect of treatment type on total volume of biogas	**Effect of bottle number on total volume of biogas**
Not significant for C+LD.A compared to C+LD.B (P = 1.000)	Not significant (P > 0.05)
Not significant for C+LD.A or C+LD.B compared to controls (P = 0.884)	

Table 3.23 Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for total biogas in trial 3. Factors tested were treatment type and bottle number. (Treatment types were controls; co-digestion of creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and co-digestion of creamery waste and 2 g/L *Laminaria digitata* (C+LD.B). The response was total volume of gas. The significance of the effect is indicated by the adjusted P-value

Due to readings being affected by backflow, it is difficult to judge the actual difference in gas production between digester groups in trial 3. There were only small differences between total volumes from different group of digesters. Different amounts of water and permanganate were removed from or pushed back towards the scrubbers and cylinders. There were several negative readings for biogas production. On average, readings from the different digesters tended to go in the same direction. Figure 3.14 shows some of the permanganate and water remaining in the set-up at the end of trial 3. There were different levels of influx into the bottles, and contents varied in colour (figure 3.15). Digester samples at the end of trial 3 seemed still to contain some gas bubbles, although these may have been from proteins. When the digester caps were removed following final readings, there was a flurry of bubbles in several of the cylinders, although this may have been at least partly air.

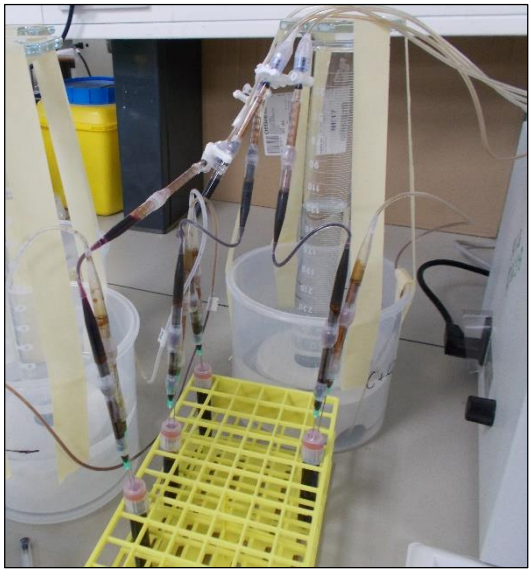

Figure 3.14 Photograph of backflow of potassium permanganate and water remaining in part of the anaerobic digester set-up at the end of trial 3

Figure 3.15 Photograph of anaerobic digester bottles after trial 3. Backflow of potassium permanganate and water during the trial changed colouring. Left to right are control 1, control 2, and control 3, C+LD.A1, C+LD.A2 and C+LD.A3, and C+LD.B1, C+LD.B2 and C+ LD.B3

Figure 3.16 shows the mean biogas produced per day by each digester type in trial 3. Table 3.24 shows the mean maximum daily volume of gas produced by each of the groups of digesters in trial 3, and the day on which each maximum was reached. The highest rate of biogas production for each of the controls was seen between day 1 and day 2. As was seen in the controls, the highest rate of production for each of the C+LD.A digesters was between day 1 and day 2, although C+LD.A1 had the same rate of production between day 4 and day 5. Like the C+LD.A digesters and the controls, C+LD.B1, C+LD.B2 and C+LD.B3 each had their highest rate of biogas production between day 1 and day 2 (although C+LD.B2 had the same rate between day 4 and day 5). One-way ANOVA with Tukey pairwise comparison showed no significant difference between the maximum rates for the controls and C+LD.A ($P = 0.067$) or for the controls and C+LD.B ($P = 0.294$). The difference between C+LD.A and C+LD.B was not significant ($P = 0.725$).

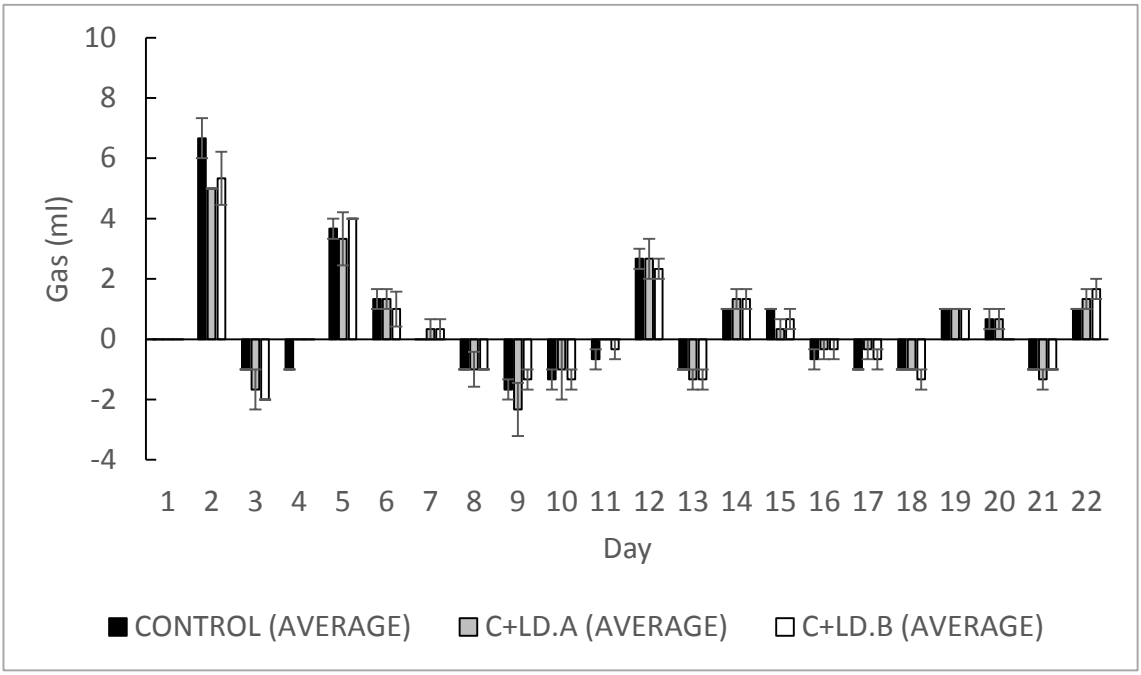

Figure 3.16 Bar chart showing the mean biogas production per day (with mean standard error) from the controls; the digesters with creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and the digesters with 2 g/L *Laminaria digitata* (C+LD.B), in trial 3. (All groups of digesters reached their mean maximum daily production on day 2. The results were severely affected by backflow)

Mean maximum daily gas volume (ml) with mean standard error (SE)		
Controls (1, 2 and 3)	**C+LD.A (1, 2 and 3)**	**C+LD.B (1, 2 and 3)**
6.67 ± 0.67 (Day 1-2)	5.00 ± 0.00 (Day 1-2)	5.33 ± 0.88 (Day 1-2)

Table 3.24 Results for mean maximum daily biogas volume produced by the control digesters; the digesters with co-digested creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and the digesters with co-digested creamery waste and 2 g/L *Laminaria digitata* (C+LD.B) in trial 3. The days corresponding to each maximum are shown

Table 3.25 shows the results of ANOVA and Tukey analysis relating to the effect of treatment type and bottle number on daily biogas volume in trial 3. The full results of these analyses on daily gas are in appendix 7.

Effect of treatment type on daily volume of biogas	**Effect of bottle number on daily volume of biogas**
Not significant (P > 0.05 for all differences of means)	Not significant (P > 0.05)

Table 3.25 Results of analysis of variance (ANOVA, general linear model) and Tukey analysis for daily biogas in trial 3. Factors tested were treatment type and bottle number. (Treatment types were controls; co-digestion of creamery waste and 1 g/L *Laminaria digitata* (C+LD.A); and co-digestion of creamery waste and 2 g/L *Laminaria digitata* (C+LD.B)). The response was total volume of gas. The significance of the effect is indicated by the adjusted P-value

3.5 Testing of null hypotheses

Due to changes in the inoculum between trials, each trial must be treated as a separate experiment, relative to controls. Where ANOVA and Tukey analysis have been done on controls and fed digesters within the same trial, the resulting adjusted P-values can be compared between trials. A lower P-value will indicate a more significant effect. Significance is judged at 95 % probability of being different from zero. The null hypotheses are tested below.

- *Null hypothesis 1*

  The addition of macroalgae to inoculum will have no effect on the total volume of biogas produced in anaerobic digestion batch trials, as compared with the total volume of biogas produced from inoculum only.

Results from trial 1 (section 3.2.6) showed that the addition of macroalgae to inoculum significantly increased the total volume of biogas (P = 0.021). $H_0$1 can therefore be rejected.

- *Null hypothesis 2*

  The addition of macroalgae plus sewage sludge pellets to inoculum will have no effect on the total volume of biogas produced in anaerobic digestion batch trials, as compared with the total volume of biogas produced from macroalgae added to inoculum.

Results from trial 2 (section 3.3.6) showed that the addition of macroalgae plus sewage pellets to inoculum decreased total gas volume, but not significantly, relative to controls. The P-value was 0.119). Results from trial 1 (section 3.2.6) showed that macroalgae increased total volume relative to controls. The P-value was 0.021. The addition of macroalgae therefore had the greater effect. In effect, the addition of macroalgae plus pellets significantly decreased total volume relative to the result of adding macroalgae only. $H_0$2 can therefore be rejected.

- *Null hypothesis 3*

  The addition of macroalgae plus creamery waste to inoculum will have no effect on the total volume of biogas produced in anaerobic digestion batch trials, as compared with the total volume of biogas produced from inoculum only.

Results from trial 2 (section 3.3.6) showed that the co-digestion of macroalgae with creamery waste and inoculum significantly increased the total volume of gas relative to controls (P = 0.004). $H_0$3 can therefore be rejected.

- *Null hypothesis 4*

  The addition of creamery waste plus macroalgae to inoculum will have no effect on the total volume of biogas produced in anaerobic digestion batch trials, as compared with the total volume of biogas produced from macroalgae added to inoculum.

Results from trial 2 (section 3.3.6) showed that creamery waste plus macroalgae mixed with inoculum significantly increased total gas volume relative to controls. The P-value was 0.004. Results from trial 1 (section 3.2.6) showed that macroalgae significantly increased total gas volume relative to controls. The P-value was 0.021. The addition of macroalgae plus creamery waste therefore had a greater effect than addition of macroalgae alone, relative to controls. $H_0$4 can therefore be rejected. However, each addition had a significant effect and it is not known whether the difference between these effects is significant.

- *Null hypothesis 5*

  An increase in the ratio of creamery waste to inoculum will have no effect on the total volume of biogas produced in anaerobic digestion batch trials, when the same mass of macroalgae is added to this mixture of creamery waste and inoculum.

Results from trial 3 (section 3.4.6) showed that digestion of 1 g/L macroalgae with a mixture of creamery waste and inoculum, biased towards creamery waste, had no significant effect on the total biogas volume, relative to controls (P = 0.884). Results from trial 2 showed that digestion of 1 g/L macroalgae with a mixture of creamery waste and inoculum, containing a lower proportion of creamery waste than that in trial 3, significantly increased the total volume of gas relative to controls (P = 0.004). Although there was no significant difference in trial 3, the mean total volume from the digesters with macroalgae was slightly lower than that from the controls. In effect, the higher ratio of creamery waste produced significantly less gas in total than did the lower ratio. $H_0$5 can therefore be rejected.

- *Null hypothesis 6*

  An increase in the mass of macroalgae added to a mixture of creamery waste and inoculum will have no effect on the total volume of biogas produced in anaerobic digestion batch trials.

Results from trial 3 (section 3.4.6) showed that a doubling of the loading of macroalgae had no significant effect on total gas volume (P = 1.000). There was no absolute difference between mean total volumes from the fed digesters with different loadings. $H_0$6 therefore cannot be rejected. It should be noted, however, that trial 3 was the most severely affected by problems with backflow.

4 DISCUSSION AND CONCLUSIONS

4.1 The potential of co-digestion of macroalgae

There are few published studies on co-digestion of macroalgae with other substrates (section 1.4.11 and section 1.4.12). The priority in this study was that the co-digestates (*Laminaria digitata*, creamery waste and sewage sludge pellets) were relevant to the IoM. The rationale was for the null hypotheses on total biogas (section 1.7) was related to feedstock composition and solids. The success of a co-digestion can be assessed based on volatile solids (VS) reduction, total CH_4 production and CH_4 yield (e.g. Callaghan *et al.*, 1999).

The pH (8) was the same in all digester samples at the start and end of each trial, suggesting stable digestion for the 21-day duration. However, many digester samples appeared to have a pH between 8 and 9, with 8 noted as closest. A time series analysis of pH (e.g. Kuroda *et al.*, 2014) was not undertaken, so it is not known whether (or to what extent) pH changed during the course of digestion. A digester will generally self-buffer, with the system tending towards pH7 if balanced (Gerardi, 2003, cited in Redden, 2013). For the greatest biogas yield the optimal pH range in AD is 6.5 to 7.5 (e.g. Liu *et al.*, 2008). Digester pH in the current study was not adjusted and was higher than this optimum. Acids are essential for methane production and could noticeably lower the pH. The concentration of VFAs, the system alkalinity and the fraction of CO_2 in the gas will affect pH (McCarty, 1964). In a study on co-digestion of *Ulva* and dairy slurry, Allen *et al.* (2014) suggested that fresh *Ulva* could lead to rapid accumulation of VFAs, whereas drying the algae reduced this initial accumulation. The macroalgae in the current study was freeze-dried, although brown rather than green algae was used. VFAs and alkalinity were not tested. However, GC analysis of gas samples suggested a methane content of less than 40%, or less than 30% or 10% in many cases. The balance in the biogas is assumed to have been mainly CO_2. Dissolved CO_2 would lower pH, but this does not seem to have been an issue.

The pH of the macroalgae was not tested. The creamery waste had a lactic acid content of 113 mg/L (table 3.2) and a pH of 9 ± 0. However, the waste did not noticeably raise pH in digesters in which it was co-digested, even when the ratio of creamery waste to inoculum was increased. The pH was tested using test strips, which are less precise than an electrode, so pH in these digesters may have been slightly above 8. The creamery waste used in pH testing may have decomposed slightly during solids analysis and storage, before pH was tested. However, the results were comparable to the average pH of 9.1 reported by the creamery. It is likely that the more acidic inoculum has the dominant effect on pH in co-digestion. The pH of the pellets was

not tested. However, a report (table 3.1) on pellets from the same STW notes the pH as 6.2. It is therefore assumed that the pellets in the current study were more acidic than the creamery waste or the digester samples. However, although the pellets inhibited gas production, their addition did not change the start or end pH of digesters. It seems unlikely that inhibition by the pellets was due to their lower pH, although buffering may have occurred during the trial.

A pH drop can be influenced by an increase in the concentration of ammonium (NH_4^+) due to decomposition of protein (e.g. Kuroda *et al.*, 2014). Ammonia concentrations from 1.7 to 14 g/L have been reported as inhibitory to methanogenesis (Chen *et al.*, 2008). Ammonium and free ammonia were not tested in the current study. However, table 3.1 shows an ammonium-N concentration of 7030 mg/kg (dry matter basis) and a total nitrogen (Kjeldahl) concentration of 70.5 g/kg (dry matter basis). 0.5 g/L pellets were co-digested with macroalgae in trial 2. Given the above concentration, this equates to 3.52 mg/L ammonium-N added from the pellets alone. This is higher than the lower inhibitory concentration reported by Chen *et al.* (2008). Total phosphorous in the pellets is assumed to be approximately 19,500 mg/kg (dry mass basis, table 3.1). No elemental analysis was done on the inoculum. However, as the sludge was from a STW, it was likely to be rich in N and P. Table 3.1 also shows the content of various metals in the pellets. Some substrates have been noted to cause unstable digestion, related to metals content and pH equilibrium; mineral supplementation or the mixing of different substrates has been suggested in these cases (de Waart *et al.*, 1987).

Optimum production of biogas requires maintenance of a balanced C/N ratio (e.g. Deublein and Steinhauser, 2008). Chynoweth (1980) noted greater biogas production from *Laminaria* when C/N ratios were low, whereas Habig *et al.* (1984), using *Ulva* and *Gracilaria,* saw an increase in biogas production with increased C/N ratio. Given high levels of N in macroalgae, ammonia can accumulate and cause inhibition (e.g. Costa *et al.* 2012). *Ulva lactuca* has a C/N ratio of less than 10, which can cause inhibition by excess levels of TAN (Allen *et al.*, 2013).

A ratio of 20/1 to 30/1 has been suggested as best (e.g. Kelly and Dworjanyn, 2008). The C/N ratio of the macroalgae in the current study was only slightly below the lower limit (20/1) of optimum ratios suggested by Kelly and Dworjanyn (2008). Inhibition was not seen in mono-digestion of the macroalgae in trial 1, which produced significantly more biogas than the controls ($P = 0.021$). From %C and N determined in the current study, the C/N ratio of the pellets is calculated as 6.006 ± 0.008. Both %C and %N in the pellets (40.28 ± 0.06% and 6.71 ± 0.01%

respectively) were higher than in the macroalgae (30.04 ± 0.06% and 1.64 ± 0.02% respectively). However, the macroalgae had the higher C/N ratio (18.359 ± 0.203). The pellets were not mono-digested. Given the above %C and %N values, the 50/50 mixture of pellets and macroalgae in trial 2 had an overall C/N ratio of 12.18 ± 2.76. In effect, the addition of macroalgae plus pellets to inoculum significantly decreased total biogas volume relative to the result of adding macroalgae only ($P = 0.119$ and 0.021 respectively, relative to controls). This may have been due to the pellets lowering the C/N ratio and causing inhibition, although the inoculum is thought to have dominated, and its C/N ratio was not determined. A possibility could be to use a lower ratio of pellets to macroalgae. This might also dispose of a larger volume of beach-cast in commercial AD. However, the macroalgae in the trials already had a slightly lower C/N ratio than is ideal.

There were issues with analysis of VTS in the pellets in this study. Results were disregarded. However, the sludge pellets are heated at over 400°C when formed. Having undergone advanced oxidation, they would be expected to be low in VS. If the VTS content of the pellets was lower than that of the macroalgae, it could be assumed, because loadings of lyophilised mass were equal, that the pellets were problematic due to composition rather than excessive solids. Insufficient VS was likely not the main cause for the poor performance, given that solids in S+LD were still higher than in the controls.

The clear difference between gas production from macroalgae and controls in trial 1, and the stability of digestion, indicate no inhibition due to overloading. Analysis of digester samples from trial 1 showed that both SS and VSS in the controls and in the LD digesters significantly decreased by the end of the trial ($P < 0.05$). Solids therefore seem to have been converted to biogas.

The same amount of feedstock (lyophilised mass basis) was added to the same inoculum in trial 1 as in trial 2. Microbial changes occurred in the inoculum between trials, which may have affected the response of adding feedstocks. However, gas production was still relatively good where the inoculum was mixed with creamery waste.

The inoculum was not acclimated before trials. However, given its origin, it would be expected to be more adapted to sewage than to the other feedstocks. The pellets caused inhibition

despite this. It is not known whether gas production from the mixture with pellets might have changed had the trial been extended beyond 21 days.

Although it had the bacteria necessary to start the AD process, the inoculum sludge was unrepresentative of the IoM, because a digester on the island would not be treating sewage. Given the high solids content of the sludge, the trialled digestion of macroalgae and inoculum, without waste, could still be considered a co-digestion. For the purposes of the study, it was treated as mono-digestion, for comparison with the wastes. However, as no overloading or instability was apparent, this could demonstrate successful co-digestion of sludge with macroalgae. The addition of macroalgae significantly increased total gas relative to the sludge alone ($P = 0.021$). Co-digestions of macroalgae with various sludges have been undertaken elsewhere (e.g. Tedseco *et al*. 2013; Oliveira *et al.*, 2014; and Costa *et al.*, 2012), as reviewed in section 1.4.11.

Because the creamery waste was liquid with few solids, elemental analysis was not done. It was thought that even if sufficient solids could be extracted, they might not be representative. Further information on the nature of the creamery waste is given in section 2.1.3. The sample used was collected in January, when milk would be approaching a low point (around February or March) in protein. Additionally, because less cheese than usual had been produced, the sample was mostly wash water and possibly did not contain whey. Whey is rich in protein, and higher whey content might lead to more production of ammonium. In a pilot-scale study, Matsui and Koike (2010) co-digested macroalgae with milk waste. The authors noted that care must be taken in using milk as a fermentation material, because ammonia production is generally thought to cause inhibition, but that ammonia levels in their study may not have been high enough to prevent fermentation. Calcium (Ca) was not analysed in the current study but, given the nature of the creamery waste, would be expected to be present. Ca levels of 2.5 to 4 g/L have been noted as moderately inhibitory, and 8 g/L as strongly inhibitory (Chen *et al.,* 2008). The low lipid levels in macroalgae are beneficial for AD (e.g. Bruton *et al.*, 2009). Average readings from the creamery show the waste to have a fat content of 358 mg/L.

Buffering of digesters might be related to salt. The inoculum was not diluted with seawater or freshwater, but was rich in solids. The salinity results for the controls in trial 1 and trial 2 should be comparable.

The literature shows mixed results on the impact of salinity on AD of macroalgae (section 1.4.9). As there was no difference in salinity (15 ± 0.00) between the start and end or between any digester samples in trial 1, the macroalgae did not noticeably add salt to the digesters. The algae had been washed, so this is not unexpected. Beach-cast on the IoM might require washing if sand is a problem in AD. However, pre-treatment increases costs. Additionally, fermentable products may be lost along with removed salts (Kelly and Dworjanyn, 2008).

Hierholtzer and Akunna (2012) noted that tolerance to salts can be greater when levels of ammonia are low. In the current study, tolerance might therefore have been higher where there was a higher concentration of inoculum, or where pellets were added.

The salinity of the creamery waste (without inoculum) was not tested. However, the effect of the waste can be seen where it was mixed with inoculum and macroalgae, in trial 2. The initial salinity in the controls was significantly higher than that in C+LD ($P = 0.000$). The addition of creamery waste seems to have lowered the salinity, without this lowering being compensated for by any salt from macroalgae added. This is in line with the results from trial 1, in which there was no difference in salinity between the controls and LD digesters. The creamery waste might be expected to contain salt, given solids from the cheese-making facility, but the results suggest higher initial salt content in the inoculum. The creamery waste was mostly wash water (i.e. freshwater). Operational differences might give different salinities. However, salinity in the C+LD digesters, like in the controls, increased significantly from the start to the end of trial 2 ($P = 0.000$ for both). The greatest increase was from the creamery waste, and the salinity of the waste at the end was not significantly different from that in the controls ($P = 0.750$). It is possible that salt was released into solution from breakdown of solids in the waste during digestion.

In trial 2, the initial salinity in S+LD was significantly higher than in the controls ($P = 0.003$). However, in contrast to the controls and C+LD, there was a significant decrease in salinity in S+LD ($P = 0.000$) to a level significantly lower than these other digesters ($P = 0.000$). Despite the relatively high start value, the salt apparently from the pellets was therefore not in the supernatant by the end.

In trial 3, start-salinity and end-salinity were between 0 and 2‰ in all digesters. Differences were thought to indicate little. The controls and fed digesters in this trial had much more

creamery waste (and less inoculum) than the digesters in previous trials. However, as suggested by the previous trials, addition or increase in loading of macroalgae did not seem to measurably increase salinity.

There were issues with solids analysis of the lyophilised macroalgae and pellets, with possible loss of material. The methods may not have been the most appropriate for the samples. In addition, the macroalgae and particularly the pellets were hygroscopic and probably absorbed some moisture while being ground etc. The TS content of macroalgae was noted as 920,584 ± 114 mg/kg and VTS as 430,650 ± 218,566 mg/kg (46.8 ± 23.7 % in SS). The mean SE was particularly high for VTS. Mean TS (958,804 ± 649 mg/kg) in the pellets was slightly higher than for the macroalgae, but with overlap considering SE. VTS results for the pellets were disregarded. However, having undergone advanced oxidation, the pellets were expected to be low in VTS.

Mean SS content in the creamery waste was 476.0 ± 15.0 mg/L. Mean VSS content was 450.0 ± 15.2 mg/L (94.58 ± 1.71% in SS). These values are lower than the current average figure of 9000 mg/L solids provided by the creamery. Fluctuations in solids content in discharge from the creamery are expected to be reasonably predictable and shorter-lived than seasonal variations in protein content. It seems likely that solids would not reach levels sufficient to cause overloading, and changes may have little impact on digestion. In the lab trials, the loading of macroalgae was relatively low, and no overloading was apparent in mono-digestion of macroalgae with inoculum in trial 1. Analysis of the controls in trial 1 and trial 2 showed the inoculum to have a SS content of over 48,000 mg/L and a VSS content of more than 30,000 mg/L. The inoculum therefore had a much higher solids content than seems likely to be reached in the creamery waste, even if the average of 9000 mg/L in the waste is exceeded. 1 g/L (1000 mg/L) (or 2 g/L) solid feedstock added was a small mass relative to the high solids content of the inoculum.

In trial 1, controls produced a mean total of 132.33 ± 3.18 ml biogas, compared with 159.67 ± 6.69 ml from the LD digesters. The addition of macroalgae significantly increased the total ($P = 0.021$). This is in line with digester samples. Macroalgae significantly increased start-solids ($P > 0.05$), but the controls and LD were not significantly different in terms of end-solids ($P < 0.05$), suggesting that a larger quantity of solids were degraded in the fed digesters. The bacteria may be working more quickly and the change in composition may have had an effect. Daily gas

production was observed, and treatment and bottle effects were analysed, but rate curves were not investigated in detail. Composition, not just quantity of solids, may be a factor.

Hydrolysis at Bran Sands STW is done mechanically rather than using bacteria. There would be a lag before the bacteria would begin working in the digesters, and it was important not to over-feed during this initial phase as the bacteria would not work properly.

Biogas production from LD in trial 1 (day 1-2) peaked a day earlier than the controls. There seems to have been rapid initial degradation, although the controls also peaked early. This may be related to agitation. Mean daily gas production tended to decrease or remain similar from around day 5. The substrate therefore seems to have been largely used up in the early phase of the trial.

Although there were issues with backflow in trial 2, these were largely remediated by agitation, and any differences between amounts of liquid remaining in tubing were not thought to be problematic for the biogas data. In the early phase of the trial, gas took time to show in cylinders. There was some immediate backflow after gas sampling, on days 3, 7, 10 and 13. It is thought that some of the gas production suggested by the data would not have shown up had readings not been corrected for gas sampled.

Sudden, severe backflow was noted on day 3 of trial 3. Permanganate is an antibiotic and is expected to have affected the bacteria where there was influx into digesters. Liquid removed from cylinders and the lack of gas pushing through liquid in tubing into cylinders affected cylinder readings.

The decrease in gas production between trials was partly due to backflow occurring in trial 2 and trial 3. Trial 3 was most severely affected. However, part of the reason for backflow might have been that the digesters in later trials produced less gas, with lower partial pressure. The partial pressure of biogas produced was possibly insufficient for bubbles to be completely pushed through. The tubing used in the set-up had a small internal diameter (1.5 mm) and there may have been capillary problems if gas partial pressure was low. In a previous study (Redden, 2013) wider tubing was used and did not suffer the same issues. The backflow issues in the current study seemed to be related to the relative levels of the digesters and various parts of the AD set-up, and the gradients of the tubing, valves and attachments.

There were several negative readings in trial 3. Data for biogas production in this trial appears to have been affected systematically. Readings fluctuated, with those from different digesters tending to go in the same direction at the same time. Liquid moved back and forth through the set-up. The issue seems to be operational, rather than gas having been lost. Changes in atmospheric pressure seemed a likely cause of fluctuation. In trials 1, 2 and 3 respectively, mean MSLP values at the time of cylinder readings were 1013.8 ± 1.36 hPa, 1010.9 ± 1.42 hPa and 1005.70 hPa ± 1.82 hPa respectively. A control cylinder to check the effect of atmospheric pressure was not set up in trial 2 or 3, as this had already been done in trial 1. Pressure is thought to have had some effect on daily gas readings, although the correlation with the control cylinder did not suggest it was closely related. Despite MSLP being higher at points during trial 1, no backflow was observed in trial 1 (except immediately after gas was sampled). Atmospheric pressure therefore does not appear to be the cause of backflow in later trials, when movement of permanganate was frequently rapid.

The absolute differences in biogas production within trials were smaller than the differences between trials. Although agitation generally helped to released gas, particularly in trial 2, total yields declined throughout the series of three trials. It has been suggested that, wherever possible, fresh sludge should be used in batch biodegradability tests (e.g. Angelidaki *et al.,* 2009). This was not practical in the current study. Microbial changes in, and release of gas from, the inoculum during storage (between trials) were unavoidable and were accounted for in the experimental design for gas volumes. However, this storage is thought to have affected gas production and methane content. The controls in trial 1 and trial 2 were inoculum only, from the same batch. Mean total gas production from controls in trial 1 was 132.33 ± 3.18 ml. The total from those in trial 2 was much lower (32.33 ± 6.64 ml).

It is difficult to predict biogas composition before testing. However, typical output from AD would be 50-80% methane. Methane contents in this study were lower than expected, and digestion therefore needs to be optimised. Gas samples from trial 1 contained less than 30% or less than 10% methane. Samples from trial 2 had less than 40% or frequently less than 10% methane. The balance was assumed to be CO_2 with trace amounts of other gases. The samples from trial 2 did not have the same issues with air in digester tubing as were thought to have occurred in some samples from trial 1. Methane was generally lower in trial 2 despite this. No gas was sampled during trial 3, due to backflow issues. The GC replication was generally good, suggesting that the problem was not with sampling or injection. In addition to there being

microbial changes in the inoculum during storage, gas samples from trial 1 and 2 may have deteriorated slightly before analysis. The tubes used for storage were theoretically gas-tight and were stored in the dark. However, it is not known whether the bungs deteriorated after being punctured by needles. Ideally, analysis would have been done as soon as possible after sampling. The 21-day duration of trials was relatively short. An increase in methane content after the end of this period might have been seen, although gas production was generally tailing off by day 22.

Co-digestion of 1 g/L macroalgae with creamery waste and inoculum significantly increased the total volume of gas relative to inoculum only ($P = 0.004$). The addition of macroalgae to inoculum also increased total volume, relative to controls ($P = 0.021$). The macroalgae plus creamery waste had the greater effect of the two. However, the significance of the difference between the two effects is unknown. It is clear that the addition of creamery waste to inoculum enhances biogas production. The creamery waste was lower in solids and bacteria than the inoculum. Dilution with waste might therefore have been expected to reduce biogas production. The improvement created by the waste might have been due to compositional change or lowering of the solids content as compared with inoculum. Digesters with more inoculum were possibly closer to being overloaded. The viscosity of mixed creamery waste and inoculum was lower than that of inoculum only. It was noted during agitation in trial 2 that gas was released most readily from the digesters containing creamery waste. This may be part of the explanation for their relatively high production. After an initial lag, there was a sharp increase in gas production rate from these digesters.

Following the lag, the rate of production from C+LD seemed good, suggesting that sustained feeding would be of interest. The rate tailed off towards the end of the trial. This suggests that the substrate had been digested, although a second phase of digestion (of macroalgae) might have been seen had the trial been extended.

In effect, relative to controls, a higher ratio of creamery waste to inoculum ($P = 0.884$) produced significantly less gas in total than did a lower ratio of creamery waste to inoculum ($P = 0.004$), when 1 g/L macroalgae was added to the mixture. The mean total from the digesters in trial 3 (with the higher ratio of waste) was slightly lower than that from the controls. Given the earlier observations that creamery waste enhanced biogas yield, with or without macroalgae being added, an increase in the ratio of creamery waste to inoculum might have been expected to

further enhance the yield. However, trial 3 had the worst problems with backflow, which severely affected the data.

An increase from 1 g/L to 2 g/L in the macroalgae co-digested with creamery waste and inoculum had no significant effect on total gas volume, (P = 1.000). The increase in substrate available for digestion might have been expected to produce more gas or cause overloading. Overloading does not seem likely. 2 g/L is a reasonable loading, and the creamery waste-inoculum mixture in trial 3 had a lower solids content than mixtures or pure inoculum in trial 1 or trial 2. The effect of potassium permanganate influx on the digestion in trial 3 is unknown. Some gas continued to be produced.

Backflow may have been due to insufficient loading of feedstock**.** The digesters were in batch mode rather than being fed at intervals. It is possible that suction occurred as the bacteria initially used up the substrate. Each trial had a different amount of time between addition of sodium acetate (to test for methanogens) and the start of the trial (when all feedstocks were added and digesters connected to cylinders). As noted, based on the pH of 8, dissolved CO_2 does not seem to have been the cause of the backflow.

It is decided that the pellets can be ruled out as a potential feedstock for co-digestion with macroalgae. This is based largely on their poor performance in the digestion trials, although use of an alternative inoculum might improve results. Another factor is that the pellets are less in need of a disposal route than is the creamery waste. The pellets currently go to the EfW plant. Creamery waste is discharged into the sea and the licence for discharge expires in October 2019. Co-digestion with creamery waste could potentially enhance biogas production from macroalgae, with the added benefit of utilising waste as a resource.

4.2 Suggestions for further work (experimental)

4.2.1 Design of anaerobic digesters

The digester set-up in the current study suffered issues with liquid backflow. Wider tubing might help to prevent any capillary issues. Gradients or relative heights of parts of the set-up could be adjusted if the hydrostatic head was an issue. Although the headspace in the H_2S scrubbers was minimised, small amounts of gas were sometimes trapped in the tops. Given the low level of H_2S production, the scrubbers could probably be eliminated. If backflow of water was still an issue, check valves could be used. Agitation should be comparable between trials.

Although it would make for a more costly and complicated set-up, agitation ideally would be continual (section 1.4.4). For reasons outlined in section 2.2.5, the digester headspaces were not flushed (e.g. with air or nitrogen). However, flushing might help to ensure that they were anaerobic. There are several alternative (volumetric and manometric) methods of measuring biogas production (e.g. Raposo *et al.,* 2011). The method in the current study was relatively crude but expected to be sufficiently reliable. Others might simplify gas sampling and analysis, or improve the overall results.

4.2.2 Experimental design

Controls

Given constraints on equipment and space, a limited number of digesters was included in each trial. Due to microbial changes in the inoculum, each trial was assessed as a separate experiment, based on controls. However, direct comparison of more digester types within the same trial might be useful.

Gas sampling

The gas sampling could be improved, possibly including different days and the end of each trial. It might be useful to vary the intervals between sample times, according to activity levels during digestion (e.g. Shi *et al.*, 2013).

Retention time and organic loading

The retention time (21 days) in the current study was relatively short. This could be extended in further trials. Short experimental times do not allow forecasting of long-term accumulation of inhibitors (e.g. Allen *et al.*, 2014). Additionally, two-phase digestion (of mannitol and laminarin followed by alginate) has been seen in previous studies on *Laminaria saccharina* (Østgaard *et al.*, 1993) and *L. hyperborea* (Horn and Østgaard, 2001).

In the current study, co-digestion was the main focus, but further trials could compare the effects of a range of feedstock masses on biogas production. Testing (laboratory- and pilot-scale) of optimum and maximum OLR for substrates in co-digestion is important for the design and upgrading of biogas plants (Ganesh *et al.,* 2015).

Feedstocks could be added on the basis of VS rather than lyophilised mass, if solids were determined accurately. In the current study, the focus was on batch AD, but feeding at intervals

would be of interest. Dose response could be tested and stable production compared with static production. It seems likely that a larger mass could be added without overloading the digesters.

Ratios of co-digestates

As was done with macroalgae in the current study, wastes could be digested individually (with inoculum only) for comparison with co-digestion. Different ratios of macroalgae and waste in co-digestion could be tested. This might indicate whether or not the effect of co-digestion is additive. Linearity would indicate a lack of synergy. Co-digestate ratios could be considered in terms of modifying C/N ratios for prevention of inhibition and optimisation of AD (section 1.4.2). In this study, macroalgae was combined with one waste per co-digestion. Co-digestion of macroalgae with two or more wastes together could be investigated, considering quantities and variation in the waste streams (section 4.3.2). Potential co-digestates other than creamery waste and sewage pellets are suggested in section 4.2.6.

4.2.3 Inoculum

Source

The digestion trials might be improved by the use of inoculum from a source more representative of the IoM (i.e. non-sewage).

Concentration and ratio to substrate

Ideally, the inoculum would contain fewer solids than that used in the current study. The retention time (section 4.4.2) could be extended to allow for any lag phase in biogas production. However, there is probably scope to reduce solids and still provide data within a reasonable timeframe. Although feedstock loading could be investigated (section 4.2.2), optimal loading might depend partly on the solids already in the inoculum. The influence of the inoculum/substrate ratio on biodegradation tests is unclear (Raposo *et al.*, 2011).

Bacteria

It has been suggested that fresh sludge should be used when possible (Angelidaki *et al.*, 2009). In the current study, microbial changes in the stored inoculum were accounted for by controls. However, the effect on biogas volume and methane content is unknown. A small amount of inoculum from each trial could be frozen and phylogenetic analyses undertaken using ribosomal RNA (rRNA).

Sludge would ideally be acclimated by feeding over a long period with a mixture of the macroalgae and wastes. This could be done on a volume basis and the solids tested. A mixture of bacteria adapted to all feedstocks should be created so that there is no lag phase in digestion.

4.2.4 Analyses

Chemical analyses

For investigation of controls on AD, digester samples and feedstocks could be analysed for VFAs (using GC), soluble saccharides (e.g. by hot water extraction, Karsten *et al.*, 1991), total proteins (e.g. using various solutions and solvents, as adapted by Redden (2013) from standard methods (Kochert, 1978)), and COD (using a strong oxidising agent and acid). Accumulation of ammonia can inhibit AD (section 1.4.9, e.g. Costa *et al.*, 2012). Ammonia was not tested in this study, but could be an area for further work, particularly in relation to C/N ratios of feedstocks. S content of substrates and H_2S content of biogas could be determined. H_2S is discussed further in section 4.2.7. CO_2 in biogas could also be measured. Additional analyses related to process stability could include trace elements and heavy metals (e.g. Allen *et al.*, 2014). Test strips were thought adequate for determining pH in the current study. However, given a different set-up, digester pH could possibly be monitored more precisely (using an electrode) and as a time series.

Rate of biogas production

Further work might investigate the rate constant (k) for biogas production. Given an equation relating biogas production to biomass and time, the goodness of fit of the observed data could be determined. The value for k would suggest whether an increase in biogas production was due to an increase in biomass or in the rate at which the bacteria were working. Subdivision of the organic loading between feedstocks would indicate whether composition or just solids quantity was a factor.

4.2.5 Macroalgae

Pre-treatment

The methodology might be improved in order to retain as many components of the macroalgae as possible. The current study used dry material, and a better method might have been to not chop the algae but dry it whole before grinding. However, using wet material, the effects of various pre-treatments (such as washing, maceration or ensilage) on biogas production could be investigated, and commercial costs considered. Pre-treatment is discussed in section 1.4.2.

Work on ensilage is currently underway at Durham University, for example (Redden *et al.*, manuscript in preparation, 2016).

Alginate extraction

Kerner *et al.* (1991) trialled AD of residues from alginate extraction from *Laminaria digitata* and *Ascophyllum nodosum*. *Laminaria sp.,* as used in the current study, are a good source of high-quality alginate (e.g. McHugh, 2003), although this idea is less relevant if beach-cast is used. Residues could be trialled in mono- and co-digestion. Commercial alginate extraction is a relatively simple, inexpensive process that could potentially bring additional revenue to the IoM. However, the economics must be considered (e.g. Lewis *et al.*, 2011). The biorefinery concept is mentioned in section 1.4.14. Transportation and the large volumes of water required for alginate extraction are some potential problems. Lab-scale extraction was attempted in the current study. A simplified methodology using sodium carbonate (adapted from Larsen *et al.*, 2003) was followed, as the residue (not alginate) would have been needed for digestion. However, given the mass of residue required (following drying) for digestion trials, and its lower priority, it was not used. Scale-up was problematic and would require development. The full methodology (adapted from Larsen *et al.*, 2003) could be applied to a small amount of macroalgae to in order to obtain alginate for assay.

4.2.6 Alternative wastes

There are few published studies on co-digestion of macroalgae with other substrates. The priority here is that the feedstocks tested are relevant to the IoM.

The IoM has several wastes, besides creamery waste and sewage pellets, that might be suitable for co-digestion with macroalgae. Laboratory-scale co-digestion with each of the below could be trialled, and issues including possible pre-treatment considered.

Meat-processing waste

The meat-processing factory on the IoM is next-door to the creamery and has expressed interest in developing a process such as AD (R. Bujko, pers. comms, 2014). One problem is that the waste could add undesirable substances to the AD process. Some of the biogas produced by Northumbrian Water Ltd. is used to sterilise its waste from AD. Hamawand (2015) reviewed AD in relation to the meat industry. Ramanathan *et al.* (2013) co-digested four species of

macroalgae with a mixture of slaughterhouse waste and cow dung (or with a mixture of slaughterhouse waste, cow dung and cyanobacteria).

Food waste

Much of the food brought onto the IoM is near its sell-by date. There is a high percentage of food waste on the island (A. Donnelly, pers. comms, 2014). Currently, this waste goes to the EfW plant. It could potentially be co-digested with macroalgae, although an alternative might be to redistribute the food. Like meat-processing waste, food waste could have undesirable components. Food waste is likely to vary in composition, although some of this variation might be 'diluted' by mixing with macroalgae. Lewis *et al.* (2011) assessed a number of scenarios for commercial co-digestion of macroalgae and food waste, and scoped small-scale, distributed AD as economically feasible and suitable for immediate implementation.

Brewery waste

Spent grains from brewing on the IoM are used as cattle feed and therefore seem to have little potential for AD. However, there is also discharge of liquid brewery waste containing large amounts of beer.

Wood waste

Other possible co-digestates on the IoM include woodchip cattle-stand waste (which is N-rich) and bark products from the Forestry Commission.

Waste frying oil

Co-digestion with waste frying oil has been shown to improve the BMP and methane production rate of *Sargassum sp.* (Oliveira *et al.*, 2015). This might form a waste on the IoM.

4.2.7 Hydrogen sulphide

Sulphur can inhibit methanogenesis (section 1.4.9). H_2S (produced from S) is toxic to workers on an AD plant and increases corrosion of equipment. Scrubbers add to commercial costs. H_2S was scrubbed in the present study, but not quantified or tested for. Further studies might determine the S content of feedstocks (section 4.2.4) and the relative amounts of H_2S produced. Potential H_2S concentrations in biogas can be predicted from the carbon sulphur (C/S) ratio of the substrate (e.g. Allen *et al.*, 2014). AD trials using macroalgae showed that a

ratio lower than 40 will tend to lead to larger amounts of H_2S accumulating (Peu et al., 2012). Lead acetate test strips, for example, could be used to test biogas samples for H_2S.

4.3 Wider issues: the Isle of Man as a case study

4.3.1 Supplies of macroalgae

An estimate needs to be made of the total yield of macroalgae that could potentially be used in AD. Wild harvest has been ruled out as an option for the IoM. The potential environmental impact of utilising each of the two possible supplies, beach-cast or cultivated, must be assessed. Stakeholder perceptions of macroalgae bioenergy are being considered (pers. comms, E.F. Greenwell, 2014).

Beach-cast macroalgae

Macroalgae has a low energy density and the costs of its removal must be considered. However, on the IoM, Douglas Borough Council already pays for beach-cast material to be moved downshore using a tractor or beach-rake. Large amounts of macroalgae washed up are often thought to be unsightly, and decomposition can cause odour problem as well as large hatches of seaweed flies. Disposal options have been considered.

A large and important uncertainty in assessing the feasibility of biogas production is the volume of beach-cast macroalgae. Information on macroalgae on Douglas beach was provided by Andy Crook and Steve Jackson (IoM). A diary is completed daily by the contractor on the beach. The diary is basic, including details on tides, weather and beach conditions. However, it may give a very rough indication ('light', 'medium' or 'heavy', plus comments on specific areas) of the typical volumes of macroalgae. The beach is separated into two priority zones. Although the depths of macroalgae can be approximated, there is no standard method of measurement. Depths and length of spread on the beach can vary. A tractor-load is probably around 0.5-0.75 tonnes, but the number of loads per hour would depend on several factors. The macroalgae is moved partly by the tractor and partly by the tide. Experience has shown that the tide height is a critical factor in the macroalgae being lifted from the growing beds. It is likely that heavy strandings are easiest to quantify, but these only occur a few times per year, given the right conditions. Lighter strandings tend to form smaller accumulations. Across 1 to 2 linear miles of foreshore, the volumes of these are harder to estimate. Much of the macroalgae is carried away by the next tide. Material can be re-deposited elsewhere, so double-counting would need to be avoided. One possible method could be to map the strandings when they first occur, and

make a desktop calculation of the approximate weight. However, this would depend on some assumptions regarding 'heaviness' of stranding, and the necessary time and resources are potential problems. Despite the difficulty in estimating volumes, it seems probable that the material deposited would exceed the amount that might be used in AD.

One disadvantage of beach-cast is that it is likely to contain more sulphur than cultivated macroalgae (e.g. Matsui *et al*., 2006). Additionally, unlike cultivation, the use of beach-cast does not allow selection of a particular species or strain. Sand in beach-cast may clog up the AD process. Pre-treatment (section 1.4.2 and section 4.2.5) adds to costs and must be considered relative to improvements in digestion.

The potential environmental impacts of taking macroalgae from Douglas beach must be assessed, and the relevant regulations considered. Macroalgae adds nutrients to an ecosystem and its removal could affect birds and filter-feeders, for example. Use of indicator species could be an alternative method to nutrient modelling (e.g. H. Redden, pers. comms, 2014).

Cultivated macroalgae

In aquaculture, macroalgae can be grown on ropes and harvested when needed. As noted above, cultivated macroalgae has some advantages over beach-cast. However, full-scale aquaculture is as yet unestablished on the IoM, and Douglas Borough Council is seeking a disposal route for the beach-cast macroalgae already present. The environmental impact of cultivation as compared with use of beach-cast needs to be considered. Wider ecological implications of macroalgal cultivation are discussed by Alridge *et al.* (2012), for example.

The potential growth rates of cultivated macroalgae in coastal waters around the IoM should be assessed. The island is an ideal habitat for macroalgae. Small-scale growth trials in four key sea-zone areas around its coastline have been undertaken by Durham University (H.C. Greenwell, pers. comms, 2013). Factors such as species, lifespan, seasonality and cost of harvest will need to be considered.

4.3.2 Supplies of wastes

Quantities and variation in waste streams

The total volume of waste discharged from the creamery, and any fluctuations in volume or composition, need to be assessed in relation to potential co-digestion. The licence for

discharging creamery waste into the sea expires in October 2019. If AD were to serve as an alternative disposal route, it would need to be able to handle the volumes of waste produced, unless any excess could be discharged elsewhere or stored without too much degradation. If lower volumes might be produced than would be needed for AD, a supplementary substrate (or dilution water) would need to be considered. The liquid nature of the creamery waste makes it relatively easy to handle. However, low solids content could be an issue, especially as macroalgae also have high water content. This could have implications for the AD process and for the solid digestate and supernatant produced. End-use of digestate is discussed in section 4.3.4. Protein in the milk received by the creamery peaks around October or November and has a low point around February or March. Solids (and whey) content will vary depending on operational processes. Whey in particular might affect ammonium in AD, due to its protein content. Operational processes could be further considered, in conjunction with the creamery.

Some of the above issues could be considered in assessing the potential of sewage pellets. However, it is assumed that the pellets will no longer be considered as a possible co-digestate. Other wastes for potential co-digestion are suggested in section 4.2.6. If more than one waste was to be mixed with macroalgae (simultaneously or at different times), differences between as well as within supplies of wastes would need assessment. Mono-digestion some of the time might be useful, but co-digestion might serve to even out fluctuations in supply. Macroalgae could possibly be mixed with a solid waste, with creamery waste added in place of dilution water. It has been suggested that macroalgae can be used in place of dilution water (Kuroda *et al.*, 2014). However, this would probably use less beach-cast material if AD were used as a disposal route. Ratios of different wastes in co-digestion could potentially be adjusted based on variation in supply (particularly with season). However, digester acclimation and stability would need to be considered.

Location and cost of waste disposal routes

Possible routes by which wastes might be utilised in co-digestion with macroalgae need to be studied (in conjunction with the Isle of Man Creamery Ltd. and the water and electricity authorities) and the economic implications of these routes assessed. The creamery pays a licence fee of £54,000 per annum for waste discharge. Its licence expires in 2019. The cheapest route for disposal of sewage pellets is currently the EfW plant, but it is assumed that the pellets will not be considered further for co-digestion. If alternative wastes might be used (section 4.2.6), their disposal routes also must be considered.

4.3.3 Location and cost of an anaerobic digester

It is suggested that Douglas Borough Council could be a partner in AD, but that construction of a plant would require an all-island approach (pers. comms, A. Donnelly, 2014). Various locations on the IoM need to be compared and contrasted as potential sites for an anaerobic digester. Although other factors (such as local residents and attitudes) would need to be considered, the largest cost for Douglas Borough Council is in fuels for transport, and the location of a digester would ideally minimise this. Manpower costs are fixed. Locating on Douglas quayside would be relatively expensive, but a small AD plant on Douglas beach has been suggested as ideal, with the EfW plant being another ideal location (A. Donnelly, pers. comms, 2014). The shortest distance for moving macroalgae would probably be to the EfW plant. The cost of an anaerobic digester depends partly on the size. However, much of the cost is in the compressor for gas-to-grid. The membrane for removing CO_2 adds cost. The gas-to-grid AD in Poundbury, England, vents CO_2 to the air.

4.3.4 End-use of digestate

At present there is no obvious disposal route for the residual sludge that would be produced by AD. However, a route could potentially be made. Possible disposal routes (e.g. soil amendment) and economic implications need to be considered, along with the likely volumes and composition of the sludge. Transportation is the largest cost for Douglas Borough Council. Wet sludge is problematic to transport. Supply and demand (and variation therein) for digestate must be assessed. Co-digestion in relation to N/P/K ratio and value of the digestate as fertiliser could be considered (e.g. Monnet, 2003). The amount of macroalgae or low-solids creamery waste (for example) used in AD might have implications for the volume, water content, composition and suitability of digestate produced. Potential toxicity or other environmental impacts need to be assessed. For example, heavy metals in macroalgae could be problematic (e.g. Nkema and Murto, 2013).

4.3.5 Comparison of macroalgae conversion methods

Various methods of converting macroalgae are briefly evaluated in section 1.3.6. Gasification and AD (the two technologies currently competing on the IoM) need to be assessed and compared, at least at a basic level, in terms of GHG- and energy balance. One disadvantage of AD is that the feedstock has to be diluted, and hence the process cannot deal with as much as can gasification. However, AD has the benefit of using wet macroalgae.

4.3.6 Energy landscape

The potential of the Isle of Man for development of renewable energy, specifically marine bioenergy, was introduced in section 1.5. Current supply and demand of energy on the IoM need to be assessed, using available data, in order to provide context for the potential production of biogas from AD (or gasification).

Electricity is generated in Douglas and Peel (on opposite coasts) and in Ramsey and Sulby (in smaller stations). The CCGT plant at Pulrose power station, in Douglas, is the most efficient, cost-effective large-scale source of electricity on the IoM (Cleantech Investor Ltd., 2012). At present, the biggest generator of independent power on the island is the energy-from-waste (EfW) plant, located at Douglas and managed by SITA Isle of Man (Cleantech Investor Ltd., 2012). A cable (connected to Bispham near Blackpool in north-west England) is used both to import and export electricity, although the capacity of the IoM for export is underused at present (Cleantech Investor Ltd., 2012). The island also has capacity on two gas interconnectors (one between the UK and the Republic of Ireland, and the other between Scotland and Ireland).

The Manx Electricity Authority (MEA) recently merged with the sewage works on the IoM and aims to reduce peak energy demand. Storing and then releasing energy would be ideal. Micro-hydro generation has been suggested, and salt mines on the IoM could potentially be used in pump storage. A hydrocarbon scheme has been highlighted in the 2020 vision. Some sites are now economic to explore, having not been so previously (R. Bujko, pers. comms, 2014). Energy and waste management would ideally be integrated.

Douglas Borough Council aims to reduce waste going to the EfW plant, whereas the plant operators want as much waste as possible. In a few years, the gate fee will be reasonably high. The council aims for cost-neutrality and environmental assistance, with anything additional to these being a bonus. A successful AD project would benefit the council, minimise the environmental impacts of wastes, and save money for the creamery etc. (A. Donnelly, pers. comms, 2014).

4.3.7 End-use of biogas

There are three potential end-points for the biogas produced by AD of macroalgae on the IoM. The two main options are to inject it directly to grid or to liquefy it for use as a transport fuel. The third option is to use a gas engine to produce electricity, but these engines are only around

30% energy-efficient. Several gas-to-grid projects exist elsewhere, such as Poundbury (run by J.V. Energen) and Northumbrian Water Ltd. The efficiency with which biogas could be liquefied and used in transport is another issue to consider.

4.4 Conclusions

In anaerobic digestion batch trials, *Laminaria digitata* digested with inoculum produced 159.67 ± 6.69 ml biogas per g lyophilised mass added. The addition of macroalgae significantly increased the total volume of gas, relative to controls. Relative to controls, co-digestion of macroalgae and sewage pellets had no significant effect on total gas. However, this co-digestion significantly decreased total gas relative to macroalgae only. This may have been due to a low C/N ratio. Co-digestion of macroalgae with a 50/50 mixture of creamery waste and inoculum produced significantly more gas than controls. The same co-digestion had a greater effect than digestion of macroalgae without creamery waste, although the significance of the difference between these effects is unknown. A 50/50 mixture of creamery waste and inoculum produced significantly more gas in total than did a higher ratio of creamery waste to inoculum, when macroalgae was co-digested with the mixture. An increase from 1 g/L to 2 g/L lyophilised mass of macroalgae added to mixed creamery waste and inoculum had no significant effect on total gas. However, the third trial was severely affected by liquid backflow in the digester set-up. The decline in gas yields throughout the trials is thought to have been due to microbial changes in the inoculum. Methane content in the biogas was low and would require optimisation.

The sewage pellets were suggested to be unsuitable for co-digestion with macroalgae. Creamery waste showed good potential for co-digestion. Several areas for further experimental work were identified. Additional considerations relate to the potential for, and impact of, macroalgae bioenergy on the Isle of Man.

APPENDICES

Appendix 1 Salinity results

Treatment type	Bottle no.	Sample no.	Start-salinity (‰)	End-salinity (‰)
CONTROL	1	1	15.00	15.00
CONTROL	1	2	15.00	15.00
CONTROL	1	3	15.00	15.00
CONTROL	2	1	15.00	15.00
CONTROL	2	2	15.00	15.00
CONTROL	2	3	15.00	15.00
CONTROL	3	1	15.00	15.00
CONTROL	3	2	15.00	15.00
CONTROL	3	3	15.00	15.00
LD	1	1	15.00	15.00
LD	1	2	15.00	15.00
LD	1	3	15.00	15.00
LD	2	1	15.00	15.00
LD	2	2	15.00	15.00
LD	2	3	15.00	15.00
LD	3	1	15.00	15.00
LD	3	2	15.00	15.00
LD	3	3	15.00	15.00

Results of salinity analyses of digester samples from trial 1

Treatment type	Bottle no.	Sample no.	Start-salinity (‰)	End-salinity(‰)
CONTROL	1	1	10.00	17.00
CONTROL	1	2	12.00	17.00
CONTROL	1	3	12.00	15.00
CONTROL	2	1	10.00	15.00
CONTROL	2	2	10.00	16.00
CONTROL	2	3	10.00	15.00
CONTROL	3	1	10.00	15.00
CONTROL	3	2	12.00	15.00
CONTROL	3	3	12.00	15.00
C+LD	1	1	4.00	15.00
C+LD	1	2	4.00	15.00
C+LD	1	3	4.00	16.00
C+LD	2	1	3.00	15.00
C+LD	2	2	4.00	15.00
C+LD	2	3	5.00	15.00
C+LD	3	1	4.00	16.00
C+LD	3	2	4.00	16.00
C+LD	3	3	4.00	16.00
S+LD	1	1	12.00	7.00
S+LD	1	2	12.00	7.00
S+LD	1	3	12.00	8.00
S+LD	2	1	13.00	7.00
S+LD	2	2	13.00	7.00
S+LD	2	3	12.00	8.00
S+LD	3	1	12.00	7.00
S+LD	3	2	12.00	7.00
S+LD	3	3	12.00	7.00

Results of salinity analyses of digester samples from trial 2

Treatment type	Bottle no.	Sample no.	Start-salinity (‰)	End-salinity (‰)
CONTROL	1	1	1.00	1.00
CONTROL	1	2	0.00	1.00
CONTROL	1	3	0.00	1.00
CONTROL	2	1	0.00	1.00
CONTROL	2	2	0.00	1.00
CONTROL	2	3	0.00	1.00
CONTROL	3	1	0.00	2.00
CONTROL	3	2	0.00	2.00
CONTROL	3	3	0.00	1.00
C+LD.A	1	1	0.00	2.00
C+LD.A	1	2	0.00	2.00
C+LD.A	1	3	0.00	2.00
C+LD.A	2	1	2.00	2.00
C+LD.A	2	2	2.00	2.00
C+LD.A	2	3	1.00	2.00
C+LD.A	3	1	1.00	2.00
C+LD.A	3	2	0.00	2.00
C+LD.A	3	3	1.00	2.00
C+LD.B	1	1	1.00	2.00
C+LD.B	1	2	1.00	2.00
C+LD.B	1	3	1.00	2.00
C+LD.B	2	1	1.00	2.00
C+LD.B	2	2	1.00	1.00
C+LD.B	2	3	1.00	2.00
C+LD.B	3	1	2.00	2.00
C+LD.B	3	2	2.00	2.00
C+LD.B	3	3	2.00	2.00

Results of salinity analyses of digester samples from trial 3

Appendix 2 Solids results

Treatment type	Bottle no.	Sample no.	Start-SS (mg/L)	Start-VSS (mg/L)	End-SS (mg/L)	End-VSS (mg/L)
CONTROL	1	1	47750.00	30750.00	47900.00	29950
CONTROL	1	2	50200.00	32500.00	48000.00	29650
CONTROL	1	3	53500.00	34550.00	50200.00	31150
CONTROL	2	1	49300.00	31600.00	48250.00	30100
CONTROL	2	2	49850.00	32000.00	45300.00	28450
CONTROL	2	3	52000.00	33650.00	49350.00	31150
CONTROL	3	1	49700.00	32600.00	43350.00	27650
CONTROL	3	2	52100.00	33750.00	46600.00	29200
CONTROL	3	3	48300.00	31350.00	51200.00	32150
LD	1	1	53100.00	34400.00	50500.00	31850
LD	1	2	52550.00	34050.00	48250.00	30100
LD	1	3	53650.00	34700.00	46100.00	28650
LD	2	1	50600.00	32650.00	51850.00	32550
LD	2	2	56250.00	36350.00	55550.00	34750
LD	2	3	51500.00	33450.00	47750.00	29850
LD	3	1	55250.00	36000.00	50250.00	31700
LD	3	2	49550.00	31950.00	46500.00	28750
LD	3	3	57700.00	38150.00	44700.00	27700

Results of solids analyses of digester samples from trial 1

Treatment type	Treatment no.	Bottle no.	Sample no.	Start-SS (mg/L)	Start-VSS (mg/L)	End-SS (mg/L)	End-VSS (mg/L)
CONTROL	1	1	1	51450	31950	48650	30250
CONTROL	1	1	2	47450	30050	46500	29300
CONTROL	1	1	3	49550	31650	45250	28700
CONTROL	1	2	1	50800	31900	49500	31200
CONTROL	1	2	2	46650	29300	44950	28300
CONTROL	1	2	3	48850	31000	51800	32300
CONTROL	1	3	1	53450	34050	50650	31950
CONTROL	1	3	2	52950	33850	47700	29950
CONTROL	1	3	3	49850	31350	46850	29500
C+LD	2	1	1	27300	17500	22300	14300
C+LD	2	1	2	24800	16250	23550	14900
C+LD	2	1	3	26300	17150	22950	14500
C+LD	2	2	1	26750	17300	20500	12550
C+LD	2	2	2	27500	17700	22750	14300
C+LD	2	2	3	26600	17500	23750	15250
C+LD	2	3	1	24300	15650	21000	13100
C+LD	2	3	2	25550	16400	24050	15400
C+LD	2	3	3	26200	17200	22700	14250
S+LD	3	1	1	53100	33700	44700	27350
S+LD	3	1	2	52800	33600	45750	28150
S+LD	3	1	3	55800	35550	44750	27700
S+LD	3	2	1	60950	38400	46150	28400
S+LD	3	2	2	53150	33850	47200	29400
S+LD	3	2	3	55100	34850	46200	28900
S+LD	3	3	1	54650	34300	45100	27900
S+LD	3	3	2	54050	34150	44950	27800
S+LD	3	3	3	55550	35450	44800	28100

Results of solids analyses of digester samples from trial 2

Treatment type	Treatment no.	Bottle no.	Sample no.	Start-SS (mg/L)	Start-VSS (mg/L)	End-SS (mg/L)	End-VSS (mg/L)
CONTROL	1	1	1	1050	800	1250	1100
CONTROL	1	1	2	1000	800	1300	1050
CONTROL	1	1	3	1000	*	1350	1200
CONTROL	1	2	1	1050	*	1350	1150
CONTROL	1	2	2	1250	1100	1350	1100
CONTROL	1	2	3	950	*	1250	900
CONTROL	1	3	1	1250	1150	1450	1250
CONTROL	1	3	2	1250	1050	2900	2250
CONTROL	1	3	3	1000	*	1750	1400
C+LD.A	2	1	1	1600	1500	1500	1050
C+LD.A	2	1	2	2700	1200	1650	1350
C+LD.A	2	1	3	1450	1400	1500	1450
C+LD.A	2	2	1	1700	1600	1500	*
C+LD.A	2	2	2	1250	*	1650	1500
C+LD.A	2	2	3	1600	1450	1750	1550
C+LD.A	2	3	1	1600	1400	1600	1350
C+LD.A	2	3	2	1450	1350	1500	1250
C+LD.A	2	3	3	1450	1350	1500	1250
C+LD.B	3	1	1	1800	1700	1900	1600
C+LD.B	3	1	2	1750	1600	1750	1600
C+LD.B	3	1	3	1750	1600	1650	1500
C+LD.B	3	2	1	1850	1750	1900	1750
C+LD.B	3	2	2	1700	1650	1750	1650
C+LD.B	3	2	3	1650	1600	1750	1500
C+LD.B	3	3	1	2050	1800	1950	1700
C+LD.B	3	3	2	1650	1500	1700	1450
C+LD.B	3	3	3	1600	1550	1900	1850

Results of solids analyses of digester samples from trial 3. * indicates a sample eliminated due to VSS being in excess of SS

Appendix 3 MSLP results

Day	MSLP in trial 1 (hPa)	MSLP in trial 2 (hPa)	MSLP in trial 3 (hPa)
1	1008	999	1024
2	1003	1008	1012
3	1009	1016	1013
4	1012	1012	1013
5	1014	998	997
6	1018	1007	991
7	1018	1012	988
8	1017	1014	991
9	1021	1005	1006
10	1026	1009	1008
11	1022	1014	1011
12	1019	1015	1009
13	1014	1007	1009
14	1014	1013	1004
15	1001	1013	1001
16	1004.5	1005.5	1006
17	1012	1003	1003
18	1010	1010	1003
19	1015	1015	1006
20	1012	1019	1005
21	1013	1023	1017
22	1022	1023	1009

Mean sea-level pressure readings at the times of cylinder readings in anaerobic digestion trials

Appendix 4 Biogas results (cumulative)

Day	Control 1 total gas (ml)	Control 2 total gas (ml)	Control 3 total gas (ml)	LD1 total gas (ml)	LD2 total gas (ml)	LD3 total gas (ml)
1	0	0	0	0	0	0
2	12	11	20	29	32	29
3	50	43	44	33	57	52
4	57	54	59	51	82	69
5	77	66	71	73	105	90
6	93	80	86	89	118	107
7	105	90	97	104	128	119
8	112	99	106	117	140	127
9	118	103	112	123	147	131
10	122	108	117	131	153	135
11	128	114	122	142	160	141
12	133	120	126	147	165	146
13	135	123	129	151	169	150
14	139	127	133	155	173	155
15	138	126	132	155	172	154
16	138	127	132	155	172	153
17	140	128	134	157	173	154
18	136	124	130	153	172	151
19	138	127	132	155	172	153
20	138	127	132	155	172	153
21	140	128	134	155	173	153
22	138	127	132	154	173	152

Cumulative biogas production from each digester in trial 1

Day	Control 1 total gas (ml)	Control 2 total gas (ml)	Control 3 total gas (ml)	C+LD1 total gas (ml)	C+LD2 total gas (ml)	C+LD3 total gas (ml)	S+LD1 total gas (ml)	S+LD2 total gas (ml)	S+LD3 total gas (ml)
1	0	0	0	0	0	0	0	0	0
2	10	12	10	8	8	8	10	10	9
3	9	11	10	6	8	8	9	10	8
4	10	12	10	7	8	8	9	10	9
5	11	13	12	8	10	10	11	11	10
6	12	12	12	8	9	9	10	10	10
7	12	12	12	7	8	8	10	10	9
8	15	15	15	11	12	12	13	13	13
9	15	15	15	11	12	12	13	13	13
10	15	17	16	11	13	13	13	14	13
11	17	18	18	13	11	14	16	16	15
12	17	18	18	14	11	14	15	16	15
13	18	19	18	18	12	18	16	16	15
14	20	21	19	20	17	22	16	18	16
15	20	22	20	28	25	30	16	18	17
16	23	24	22	43	39	46	19	19	19
17	26	26	22	57	53	61	19	19	19
18	27	28	22	66	59	70	19	19	19
19	28	29	21	68	60	72	18	19	19
20	31	28	20	66	59	70	18	18	18
21	37	29	20	66	59	70	18	18	18
22	44	32	21	66	59	70	18	18	18

Cumulative biogas production from each digester in trial 2

Day	Control 1 total gas (ml)	Control 2 total gas (ml)	Control 3 total gas (ml)	C+LD.A 1 total gas (ml)	C+LD.A 2 total gas (ml)	C+LD.A 3 total gas (ml)	C+LD.B 1 total gas (ml)	C+LD.B 2 total gas (ml)	C+LD.B 3 total gas (ml)
1	0	0	0	0	0	0	0	0	0
2	8	6	6	5	5	5	5	4	7
3	7	5	5	2	4	4	3	2	5
4	6	4	4	2	4	4	3	2	5
5	9	8	8	7	6	9	7	6	9
6	11	9	9	8	9	7	9	7	9
7	11	9	9	8	9	8	9	7	10
8	10	8	8	6	8	8	8	6	9
9	9	6	6	4	7	4	7	5	7
10	7	5	5	2	5	5	5	4	6
11	7	4	4	2	5	5	5	4	5
12	9	7	7	6	7	7	7	6	8
13	8	6	6	4	6	6	6	4	7
14	9	7	7	6	7	7	7	6	8
15	10	8	8	6	7	8	8	6	9
16	10	7	7	6	7	7	8	6	8
17	9	6	6	5	7	7	7	6	7
18	8	5	5	4	6	6	6	4	6
19	9	6	6	5	7	7	7	5	7
20	10	6	7	6	7	8	7	5	7
21	9	5	6	4	6	7	6	4	6
22	10	6	7	6	7	8	7	6	8

Cumulative biogas production from each digester in trial 3

Appendix 5 Biogas results (daily)

Day	Control 1 daily gas (ml)	Control 2 daily gas (ml)	Control 3 daily gas (ml)	LD1 daily gas (ml)	LD2 daily gas (ml)	LD3 daily gas (ml)
1	0	0	0	0	0	0
2	12	11	20	29	32	29
3	32	28	22	4	25	23
4	10	12	14	15	22	14
5	20	12	12	22	23	21
6	16	14	15	16	13	17
7	12	10	11	15	10	12
8	7	9	9	12	8	0
9	6	4	6	7	4	0
10	4	5	5	6	4	-1
11	6	6	5	7	6	4
12	5	6	4	5	5	0
13	2	3	3	4	4	1
14	4	4	4	4	5	0
15	-1	-1	-1	-1	-1	0
16	0	1	0	0	-1	0
17	5	4	5	5	4	4
18	-4	-4	-4	-4	-1	-3
19	2	3	2	2	0	2
20	0	0	0	0	0	0
21	2	1	2	0	1	0
22	-2	-1	-2	-1	0	-1

Daily biogas production from each digester in trial 1

Day	Control 1 daily gas (ml)	Control 2 daily gas (ml)	Control 3 daily gas (ml)	C+LD1 daily gas (ml)	C+LD2 daily gas (ml)	C+LD3 daily gas (ml)	S+LD1 daily gas (ml)	S+LD2 daily gas (ml)	S+LD3 daily gas (ml)
1	0	0	0	0	0	0	0	0	0
2	10	12	10	8	8	8	10	10	9
3	-1	-1	0	-2	0	0	-1	0	-1
4	4	4	3	4	3	3	3	3	4
5	1	1	2	1	2	2	2	1	1
6	1	-1	0	0	-1	-1	-1	-1	0
7	0	0	0	-1	-1	0	0	0	-1
8	3	3	3	4	4	4	3	3	4
9	0	0	0	0	0	0	0	0	0
10	0	2	1	0	1	1	0	1	0
11	2	1	2	2	-2	1	3	2	2
12	0	0	0	1	0	0	-1	0	0
13	1	1	0	4	1	4	1	0	0
14	2.5	5	4	5	5	7	3	5	4
15	0	1	1	8	8	8	0	0	1
16	3	2	2	15	14	16	3	1	2
17	3	2	0	14	14	15	0	0	0
18	1	2	0	9	6	9	0	0	0
19	1	1	-1	2	1	2	-1	0	0
20	3	-1	-1	-2	-1	-2	0	-1	-1
21	6	1	0	0	0	0	0	0	0
22	7	3	1	0	0	0	0	0	0

Daily biogas production from each digester in trial 2

Day	Control 1 daily gas (ml)	Control 2 daily gas (ml)	Control 3 daily gas (ml)	C+LD.A 1 daily gas (ml)	C+LD.A 2 daily gas (ml)	C+LD.A 3 daily gas (ml)	C+LD.B 1 daily gas (ml)	C+LD.B 2 daily gas (ml)	C+LD.B 3 daily gas (ml)
1	0	0	0	0	0	0	0	0	0
2	8	6	6	5	5	5	5	4	7
3	-1	-1	-1	-3	-1	-1	-2	-2	-2
4	-1	-1	-1	0	0	0	0	0	0
5	3	4	4	5	3	2	4	4	4
6	2	1	1	1	2	1	2	1	0
7	0	0	0	0	0	1	0	0	1
8	-1	-1	-1	-2	-1	0	-1	-1	-1
9	-1	-2	-2	-2	-1	-4	-1	-1	-2
10	-2	-1	-1	-2	-2	1	-2	-1	-1
11	0	-1	-1	0	0	0	0	0	-1
12	2	3	3	4	2	2	2	2	3
13	-1	-1	-1	-2	-1	-1	-1	-2	-1
14	1	1	1	2	1	1	1	2	1
15	1	1	1	0	0	1	1	0	1
16	0	-1	-1	0	0	-1	0	0	-1
17	-1	-1	-1	-1	0	0	-1	0	-1
18	-1	-1	-1	-1	-1	-1	-1	-2	-1
19	1	1	1	1	1	1	1	1	1
20	1	0	1	1	0	1	0	0	0
21	-1	-1	-1	-2	-1	-1	-1	-1	-1
22	1	1	1	2	1	1	1	2	2

Daily biogas production from each digester in trial 3

Appendix 6 Statistical analysis of biogas results (total)

Trial 1
General Linear Model: End-vol. gas (ml, day 22) versus Treatment type

Method

Factor coding (-1, 0, +1)
Rows unused 390

Factor Information

Factor	Type	Levels	Values
Treatment type	Fixed	2	Control, LD

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment type	1	1120.7	1120.67	13.61	0.021
Error	4	329.3	82.33		
Total	5	1450.0			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
9.07377	77.29%	71.61%	48.90%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	146.00	3.70	39.41	0.000	
Treatment type					
Control	-13.67	3.70	-3.69	0.021	1.00

Regression Equation

End-vol. gas (ml, day 22) = 146.00 - 13.67 Treatment type_Control + 13.67 Treatment type_LD

Comparisons for End-vol. gas (ml, day 22)

Tukey Pairwise Comparisons: Response = End-vol. gas (ml, day 22), Term = Treatment type

Grouping Information Using the Tukey Method and 95% Confidence

Treatment

type	N	Mean	Grouping
LD	3	159.667	A
Control	3	132.333	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Treatment type Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
LD - Control	27.33	7.41	(6.76, 47.90)	3.69	0.021

Individual confidence level = 95.00%

Tukey Simultaneous 95% CIs

Results of ANOVA (general linear model) and Tukey analysis showing the response of total gas with treatment type as the factor. (Trial 1)

Trial 2
General Linear Model: End-vol. gas (ml, day 22) versus Treatment type

Method

Factor coding (-1, 0, +1)
Rows unused 585

Factor Information

Factor	Type	Levels	Values
Treatment type	Fixed	3	C+LD, Control, S+LD

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment type	2	3481.6	1740.78	31.97	0.001
Error	6	326.7	54.44		
Total	8	3808.2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
7.37865	91.42%	88.56%	80.70%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	38.44	2.46	15.63	0.000	
Treatment type					
C+LD	26.56	3.48	7.63	0.000	1.33
Control	-6.11	3.48	-1.76	0.129	1.33

Regression Equation

End-vol. gas (ml, day 22) = 38.44 + 26.56 Treatment type_C+LD - 6.11 Treatment type_Control
- 20.44 Treatment type_S+LD

Comparisons for End-vol. gas (ml, day 22)

Tukey Pairwise Comparisons: Response = End-vol. gas (ml, day 22), Term = Treatment type

Grouping Information Using the Tukey Method and 95% Confidence

Treatment

type	N	Mean	Grouping
C+LD	3	65.0000	A
Control	3	32.3333	B
S+LD	3	18.0000	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Treatment Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
Control - C+LD	-32.67	6.02	(-51.16, -14.18)	-5.42	0.004
S+LD - C+LD	-47.00	6.02	(-65.49, -28.51)	-7.80	0.001
S+LD - Control	-14.33	6.02	(-32.82, 4.16)	-2.38	0.119

Individual confidence level = 97.80%

Tukey Simultaneous 95% CIs

Results of ANOVA (general linear model) and Tukey analysis showing the response of total gas with treatment type as the factor. (Trial 2)

Trial 3
General Linear Model: End-vol. gas (ml, day 22) versus Treatment type

Method

Factor coding (-1, 0, +1)
Rows unused 585

Factor Information

Factor	Type	Levels	Values
Treatment type	Fixed	3	C+LD.A, C+LD.B, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment type	2	0.8889	0.4444	0.21	0.816
Error	6	12.6667	2.1111		
Total	8	13.5556			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.45297	6.56%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	7.222	0.484	14.91	0.000	
Treatment type					
C+LD.A	-0.222	0.685	-0.32	0.757	1.33
C+LD.B	-0.222	0.685	-0.32	0.757	1.33

Regression Equation

End-vol. gas (ml, day 22) = 7.222 - 0.222 Treatment type_C+LD.A
- 0.222 Treatment type_C+LD.B
+ 0.444 Treatment type_Control

Comparisons for End-vol. gas (ml, day 22)

Tukey Pairwise Comparisons: Response = End-vol. gas (ml, day 22), Term = Treatment type

Grouping Information Using the Tukey Method and 95% Confidence

Treatment type	N	Mean	Grouping
Control	3	7.66667	A
C+LD.A	3	7.00000	A
C+LD.B	3	7.00000	A

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Treatment type Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	Adjusted T-Value	Adjusted P-Value
C+LD.B - C+LD.A	-0.00	1.19	(-3.64, 3.64)	-0.00	1.000
Control - C+LD.A	0.67	1.19	(-2.97, 4.31)	0.56	0.844
Control - C+LD.B	0.67	1.19	(-2.97, 4.31)	0.56	0.844

Individual confidence level = 97.80%

Tukey Simultaneous 95% CIs

Results of ANOVA (general linear model) and Tukey analysis showing the response of total gas with treatment type as the factor. (Trial 3)

Appendix 7 Statistical analysis of biogas results (daily)

Trial 1
General Linear Model: Gas that day (ml) versus Treatment type

Method

Factor coding (-1, 0, +1)
Rows unused 264

Factor Information

Factor	Type	Levels	Values
Treatment type	Fixed	2	Control, LD

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment type	1	9.28	9.280	0.14	0.711
Error	130	8739.35	67.226		
Total	131	8748.63			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
8.19913	0.11%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.280	0.714	8.80	0.000	
Treatment type					
Control	-0.265	0.714	-0.37	0.711	1.00

Regression Equation

Gas that day (ml) = 6.280 - 0.265 Treatment type_Control + 0.265 Treatment type_LD

Fits and Diagnostics for Unusual Observations

Obs	Gas that day (ml)	Fit	Resid	Std Resid	
7	32.00	6.02	25.98	3.19	R
73	28.00	6.02	21.98	2.70	R

202	29.00	6.55	22.45	2.76	R
268	32.00	6.55	25.45	3.13	R
271	25.00	6.55	18.45	2.27	R
277	23.00	6.55	16.45	2.02	R
334	29.00	6.55	22.45	2.76	R
337	23.00	6.55	16.45	2.02	R

R Large residual

Comparisons for Gas that day (ml)

Tukey Pairwise Comparisons: Response = Gas that day (ml), Term = Treatment type

Grouping Information Using the Tukey Method and 95% Confidence

Treatment type	N	Mean	Grouping
LD	66	6.54545	A
Control	66	6.01515	A

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Treatment type Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	Adjusted T-Value	Adjusted P-Value
LD - Control	0.53	1.43	(-2.29, 3.35)	0.37	0.711

Individual confidence level = 95.00%

Tukey Simultaneous 95% CIs

Results of ANOVA (general linear model) and Tukey analysis showing the response of daily gas with treatment type as the factor. (Trial 1)

Trial 2
General Linear Model: Gas that day (ml) versus Treatment type

Method

Factor coding (-1, 0, +1)
Rows unused 396

Factor Information

Factor	Type	Levels	Values
Treatment type	Fixed	3	C+LD, Control, S+LD

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment type	2	154.9	77.43	6.64	0.002
Error	195	2272.4	11.65		
Total	197	2427.2			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
3.41368	6.38%	5.42%	3.48%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1.997	0.243	8.23	0.000	
Treatment type					
C+LD	1.199	0.343	3.50	0.001	1.33
Control	-0.293	0.343	-0.85	0.394	1.33

Regression Equation

Gas that day (ml) = 1.997 + 1.199 Treatment type_C+LD - 0.293 Treatment type_Control
- 0.907 Treatment type_S+LD

Fits and Diagnostics for Unusual Observations

Obs	Gas that day (ml)	Fit	Resid	Std Resid	
4	10.000	1.705	8.295	2.45	R
70	12.000	1.705	10.295	3.04	R
136	10.000	1.705	8.295	2.45	R

244	15.000	3.197	11.803	3.48	R
247	14.000	3.197	10.803	3.19	R
310	14.000	3.197	10.803	3.19	R
313	14.000	3.197	10.803	3.19	R
376	16.000	3.197	12.803	3.78	R
379	15.000	3.197	11.803	3.48	R
400	10.000	1.091	8.909	2.63	R
466	10.000	1.091	8.909	2.63	R
532	9.000	1.091	7.909	2.33	R

R Large residual

Comparisons for Gas that day (ml)

Tukey Pairwise Comparisons: Response = Gas that day (ml), Term = Treatment type

Grouping Information Using the Tukey Method and 95% Confidence

Treatment type	N	Mean	Grouping
C+LD	66	3.19697	A
Control	66	1.70455	B
S+LD	66	1.09091	B

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Treatment type Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	T-Value	Adjusted P-Value
Control - C+LD	-1.492	0.594	(-2.896, -0.089)	-2.51	0.034
S+LD - C+LD	-2.106	0.594	(-3.510, -0.703)	-3.54	0.001
S+LD - Control	-0.614	0.594	(-2.017, 0.790)	-1.03	0.557

Individual confidence level = 98.08%

Tukey Simultaneous 95% CIs

Results of ANOVA (general linear model) and Tukey analysis showing the response of daily gas with treatment type as the factor. (Trial 2)

Trial 3
General Linear Model: Gas that day (ml) versus Treatment type

Method

Factor coding (-1, 0, +1)
Rows unused 396

Factor Information

Factor	Type	Levels	Values
Treatment type	Fixed	3	C+LD.A, C+LD.B, Control

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatment type	2	0.040	0.02020	0.01	0.994
Error	195	689.621	3.53652		
Total	197	689.662			

Model Summary

S	R-sq	R-sq(adj)	R-sq(pred)
1.88056	0.01%	0.00%	0.00%

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.328	0.134	2.46	0.015	
Treatment type					
C+LD.A	-0.010	0.189	-0.05	0.957	1.33
C+LD.B	-0.010	0.189	-0.05	0.957	1.33

Regression Equation

Gas that day (ml) = 0.328 - 0.010 Treatment type_C+LD.A - 0.010 Treatment type_C+LD.B
+ 0.020 Treatment type_Control

Fits and Diagnostics for Unusual Observations

Obs	Gas that day (ml)	Fit	Resid	Std Resid	
4	8.000	0.348	7.652	4.10	R
70	6.000	0.348	5.652	3.03	R
136	6.000	0.348	5.652	3.03	R

202	5.000	0.318	4.682	2.51	R
211	5.000	0.318	4.682	2.51	R
268	5.000	0.318	4.682	2.51	R
334	5.000	0.318	4.682	2.51	R
355	-4.000	0.318	-4.318	-2.31	R
400	5.000	0.318	4.682	2.51	R
532	7.000	0.318	6.682	3.58	R

R Large residual

Comparisons for Gas that day (ml)

Tukey Pairwise Comparisons: Response = Gas that day (ml), Term = Treatment type

Grouping Information Using the Tukey Method and 95% Confidence

Treatment type	N	Mean	Grouping
Control	66	0.348485	A
C+LD.B	66	0.318182	A
C+LD.A	66	0.318182	A

Means that do not share a letter are significantly different.

Tukey Simultaneous Tests for Differences of Means

Difference of Treatment type Levels	Difference of Means	SE of Difference	Simultaneous 95% CI	Adjusted T-Value	Adjusted P-Value
C+LD.B - C+LD.A	0.000	0.327	(-0.773, 0.773)	0.00	1.000
Control - C+LD.A	0.030	0.327	(-0.743, 0.803)	0.09	0.995
Control - C+LD.B	0.030	0.327	(-0.743, 0.803)	0.09	0.995

Individual confidence level = 98.08%

Tukey Simultaneous 95% CIs

Results of ANOVA (general linear model) and Tukey analysis showing the response of daily gas with treatment type as the factor. (Trial 3)

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